(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 August 2003 (14.08.2003)

PCT

(10) International Publication Number
WO 03/066806 A2

(51) International Patent Classification[7]: C12N

(21) International Application Number: PCT/US02/35501

(22) International Filing Date:
5 November 2002 (05.11.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PCT/US01/49956
6 November 2001 (06.11.2001) US
60/365,648 18 March 2002 (18.03.2002) US
60/379,188 9 May 2002 (09.05.2002) US

(71) Applicant *(for all designated States except US)*: **V.I. TECHNOLOGIES, INC.** [US/US]; 134 Coolidge Avenue, Watertown, MA 02472 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **CHAPMAN, John** [US/US]; 77 Beaconwood Road #22, Newton, MA 02461 (US). **ALFORD, Bernadette** [US/US]; 11 Carter Drive, Framingham, MA 01701 (US). **PURMAL, Andrei, A.** [US/US]; 501 Lexington Street, Apt. 9, Waltham, MA 02452 (US). **ZAVIZION, Boris** [UA/US]; 44 Broadlawn Park #20, Chestnut Hill, MA 02467 (US).

(74) Agent: **VAN AMSTERDAM, John, R.**; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report* —

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: THERAPEUTIC USE OF AZIRIDINO COMPOUNDS

(57) Abstract: Methods are provided for treatment of diseases. The methods include preventing or inhibiting transcription and/or replication of a nucleic acid molecule by administering to a subject in need of such treatment an effective amount of one or more aziridino compounds. Pharmaceutical compositions comprising the one or more aziridino compounds also are provided.

WO 03/066806 A2

THERAPEUTIC USE OF AZIRIDINO COMPOUNDS

Related Applications

This application claims the benefit under 35 U.S.C. § 119 and/or 35 U.S.C. § 365 of PCT application PCT/US01/49956, filed November 6, 2001, United States provisional application 60/365,648, filed March 18, 2002, and United States provisional application 60/379,188, filed May 9, 2002, each of which is incorporated herein by reference.

Field of the Invention

The present invention relates to the use of aziridino compounds in the treatment of disorders including infectious disease (e.g., bacterial infections, viral infections, fungal infections, parasitic infections, etc.), neoplastic disease and disorders of immune system function.

Background of the Invention

Infectious disease is one of the leading causes of death throughout the world. In the United States alone the death rate due to infectious disease rose 58 % between 1980 and 1992. During this time, the use of anti-infective therapies to combat infectious disease has grown significantly and is now a multi-billion dollar a year industry. Even with these increases in anti-infective agent use, the treatment and prevention of infectious disease remains a challenge to the medical community throughout the world. In general, there are three types of anti-infective agents, anti-bacterial agents, anti-viral agents, and anti-fungal agents, and even within these classes of agents there is some overlap with respect to the type of microorganism they are useful for treating.

Anti-bacterial agents kill or inhibit bacteria, and include antibiotics as well as other synthetic or natural compounds having similar functions. Antibiotics are low molecular weight molecules which are produced as secondary metabolites by cells, such as microorganisms. In general, antibiotics interfere with one or more bacterial functions or structures which are specific for the microorganism and which are not present in host cells. Anti-viral agents, which can be isolated from natural sources or synthesized, are useful for killing or inhibiting viruses. Anti-fungal agents are used to treat superficial fungal infections as well as opportunistic and primary systemic fungal infections.

One of the problems with anti-infective therapies is the side effects occurring in the host that is treated with the anti-infective. For instance, many anti- infective agents can kill or inhibit a broad spectrum of microorganisms and are not specific for a particular type of species. Treatment with these types of anti- infective agents results in the killing of the normal microbial flora living in the host, as well as the infectious microorganism. The loss of the microbial flora can lead to disease complications and predispose the host to infection by other pathogens, since the microbial flora compete with and function as barriers to infectious pathogens. Other side effects may arise as a result of specific or non-specific effects of these chemical entities on non-microbial cells or tissues of the host.

Another problem with wide-spread use of anti-infectives is the development of antibiotic resistant strains of microorganisms. Already, vancomycin-resistant *enterococci*, penicillin-resistant *pneumococci*, multi-drug resistant *S. aureus*, and multi-drug resistant *tuberculosis* strains have developed and are becoming major clinical problems. Widespread use of anti- infectives will likely produce many antibiotic-resistant strains of bacteria. As a result, new anti-infective strategies will be required to combat these microorganisms.

The treatment of neoplastic disease also presents challenges for the medical practitioner. New therapies have been used to target tumor blood supply (e.g., endostatin), and various types of cancers via immunological means (e.g., antibodies targeted to specific cell surface proteins, dendritic cell vaccines, and the like) and by inhibiting the enzymatic activities of specific tumors (e.g., Gleevec).

Although new therapeutic drugs and modalities have been developed, cancers frequently become resistant even to new therapies. Therefore, there still exists a need for additional therapies for effective treatment of a variety of neoplastic diseases.

Disorders of immune system function also are lacking effective treatments, or would benefit from additional or improved treatments.

Certain aziridino compounds have been proposed for the treatment of cancer. In U.S. Patents 4,233,215 and 4,704,384, aziridinylquinone compounds are proposed for therapeutic use. The patents disclosed the utility of the aziridinylquinone compounds as cancer therapeutics.

Aziridino compounds, particularly ethyleneimine oligomers, have been used for the inactivation of infectious agents in blood and blood products *in vitro*. For example, U.S. Patent 6,136,586 teaches that ethyleneimine oligomers are useful for inactivation of viruses to improve the safety of blood for use in transfusion medicine. These compounds act by

modifying nucleic acids of viruses, and other nucleic acids (e.g., in nucleated blood cells) in the blood, and thus are thought to be toxic to cells generally. Accordingly, the use of aziridino compounds is thought to be toxic to a wide range of nucleic acid-containing cells and thus unsuitable as therapeutic agents.

Summary of the Invention

It now has been discovered unexpectedly that sufficiently large doses of aziridino compounds can be administered to animals without excessive toxicity to achieve blood levels of such compounds that are effective in rendering an infectious agent, tumor cell or leukocyte innocuous. Further, it has been determined that aziridino compounds exhibit unexpectedly selective toxicity for neoplastic cells, and not normal cells. Accordingly, improved methods and products for the prevention and/or treatment of disease, particularly of infectious disease, neoplastic disease and disorders of immune system function are provided according to the invention. The results are surprising in part because the aziridino compounds act through preferential modification of nucleic acids and would not necessarily be expected to affect infectious agents, tumor cells and leukocytes without toxicity to host cells of various kinds.

According to one aspect of the invention, methods are provided for treatment of diseases. The methods include preventing or inhibiting transcription and/or replication of a nucleic acid molecule by administering to a subject in need of such treatment an effective amount of one or more aziridino compounds. In certain embodiments, the nucleic acid molecule is a non-self nucleic acid molecule. A "non-self" nucleic acid molecule is one that is not a naturally occurring nucleic acid of the subject's species (i.e., naturally occurring nucleic acid molecules of the subject's species are "self" nucleic acid molecules). In other embodiments, the nucleic acid molecule is a mutated self nucleic acid molecule. In still other embodiments, the nucleic acid molecule is a leukocytic nucleic acid molecule, which is a nucleic acid molecule of a leukocyte of the subject. Nucleic acid molecules, as used herein, include single and double-stranded RNA and DNA.

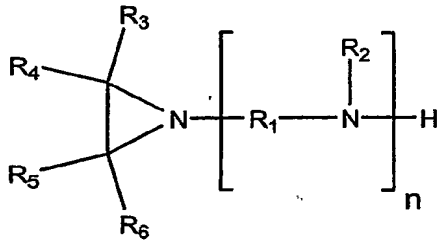
According to another aspect of the invention, methods for treating infectious disease are provided. The methods include administering to a subject in need of such treatment an amount of an aziridino compound effective to treat the infectious disease by inhibiting the growth or replication of an infectious agent that causes the infectious disease.

According to still another aspect of the invention, methods for treating neoplastic disease are provided. The methods include administering to a subject in need of such

treatment an amount of an aziridino compound effective to treat the neoplastic disease by inhibiting the growth or replication of a cell or tumor that causes the neoplastic disease, wherein the aziridino compound is not an aziridinylquinone compound.

In yet another aspect of the invention, methods are provided for treating a disorder of immune system function. The methods include administering to a subject in need of such treatment an amount of an aziridino compound effective to treat the disorder of immune system function by inhibiting the growth or replication of an immune system cell.

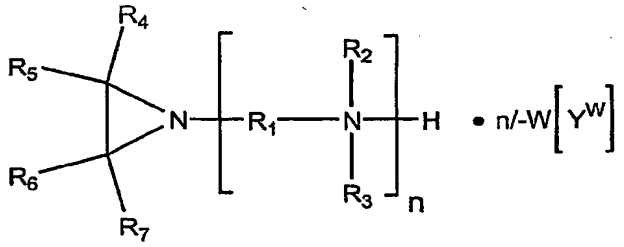
In certain embodiments of the foregoing methods, the aziridino compound contains a linear alkyl group. Preferably the aziridino compound has the structure of formula II:



(II)

wherein each R_1 is a divalent hydrocarbon moiety containing between two and four carbon atoms, inclusive; each of R_2, R_3, R_4, R_5, and R_6 is, independently, H or a monovalent hydrocarbon moiety containing between one and four carbon atoms, inclusive; and n is an integer between one and ten, inclusive. More preferably, R_2, R_3, R_4, R_5, and R_6 are H.

In other embodiments, the salt of the aziridino compound has the structure of formula III:

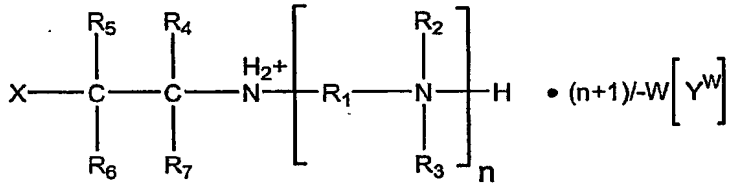


(III)

wherein each R_1 is a divalent hydrocarbon moiety containing between two and four carbon atoms, inclusive; each of R_2, R_3, R_4, R_5, R_6, and R_7 is, independently, H or a monovalent hydrocarbon moiety containing between one and four carbon atoms, inclusive; Y

is pharmaceutically acceptable counter anion; W is the valency of Y; and n is an integer between one and ten, inclusive. Preferably, R_2, R_3, R_4, R_5, and R_6 are H.

In still other embodiments, aziridino-containing compounds include open-ring counterparts to the compounds of formula (I). In one example, aziridino-containing compounds useful in the methods of the invention have the formula (IV):

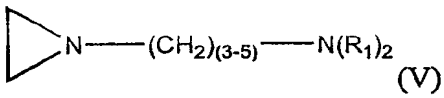


(IV)

wherein each R_1 is a divalent hydrocarbon moiety containing between two and four carbon atoms, inclusive; each of R_2, R_3, R_4, R_5, R_6, and R_7 is, independently, H or a monovalent hydrocarbon moiety containing between one and four carbon atoms, inclusive; X is Cl or Br; Y is a pharmaceutically acceptable counter anion; W is the valency of Y; and n is an integer between one and ten, inclusive.

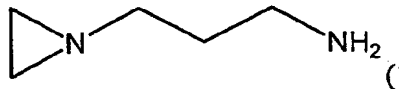
In various preferred embodiments of compounds satisfying formula (III) or formula (IV), each R_1 contains two or three carbon atoms; each of R_2, R_3, R_4, R_5, and R_6 is H; and n is one or two. Suitable counter anions include nitrate, sulfate, halide, phosphate, and tosylate ions.

In a further embodiment, the aziridino compound has the structure of formula (V):

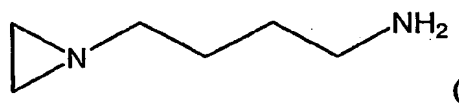


(V)

or a salt thereof, wherein each R_1 is, independently, selected from the group consisting of H, C_{1-4} alkyl, C_{2-4} alkenyl, phenyl, and benzyl. In particular embodiments, the compound is 1-aziridinepropanamine or 1-aziridinebutanamine (compounds 1 and 2, respectively):

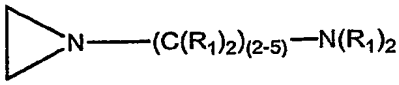


(1)



(2).

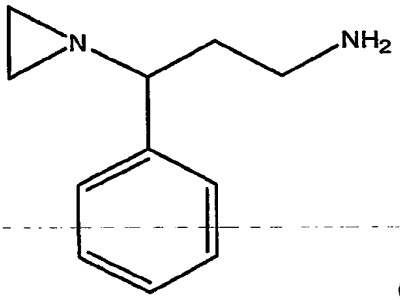
In another embodiment, the aziridino compound has the structure of formula (VI):



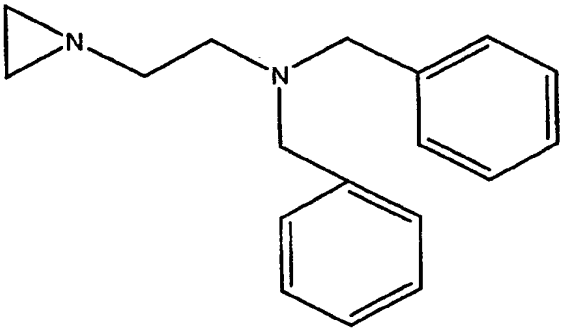
(VI)

or a salt thereof, wherein each R_1 is, independently, selected from the group consisting of H, C_{1-4} alkyl, C_{2-4} alkenyl, phenyl, and benzyl, provided that at least one R_1 is phenyl or benzyl.

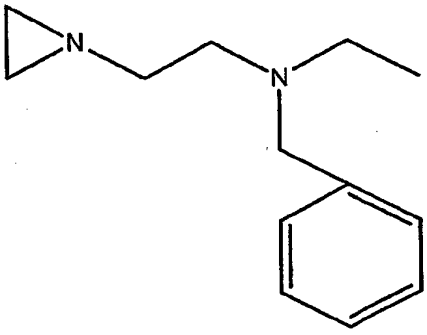
Exemplary aziridino compounds that fall within formula (VI) are 3-phenyl-1-aziridinepropanamine, N,N-dibenzyl-1-aziridineethanamine, and N-benzyl-N-ethyl-1-aziridineethanamine, and 2-benzyl-1-aziridineethanamine (compounds 3, 4, 5, and 6, respectively).



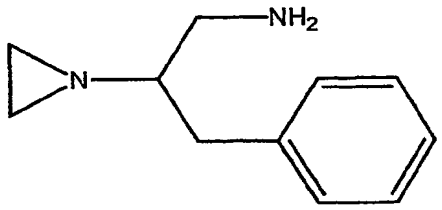
(3)



(4)

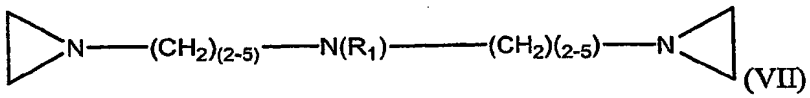


(5)



(6)

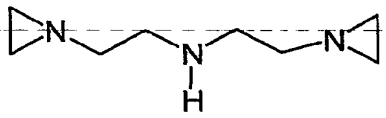
In another embodiment, the aziridino compound has the structure of formula (VII):



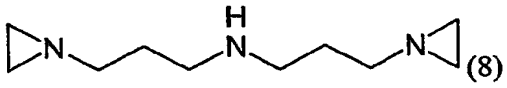
(VII)

or a salt thereof, wherein R_1 is selected from the group consisting of H, C_{1-4} alkyl, C_{2-4} alkenyl, phenyl, and benzyl.

Exemplary compounds of formula (VII) are 1,1'-[iminobis(dimethylene)]bis aziridine and 1,1'-[iminobis(trimethylene)]bis aziridine (compounds 7 and 8 respectively).

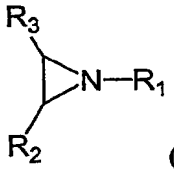


(7)



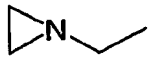
(8)

In another embodiment, the aziridino compound has the structure of formula (VIII):



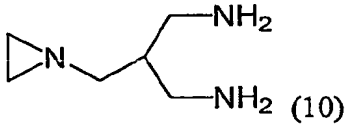
(VIII)

or a salt thereof, wherein R_1 is a C_{1-4} alkyl and R_2 and R_3 is each, independently, H or a C_{1-4} alkyl. An exemplary compound of formula (VIII) is:

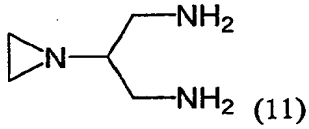


(9)

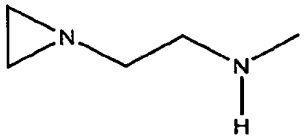
In another embodiment, the invention includes the therapeutic use of one of the following aziridino compounds:



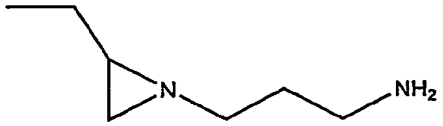
(10)



(11)



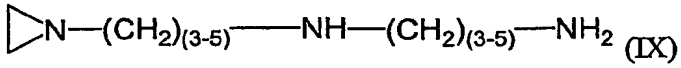
(12)



(13)

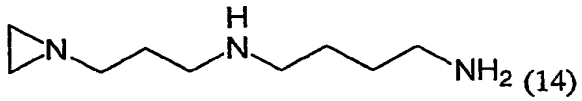
or a salt thereof.

In an additional embodiment, the aziridino compound has a structure according to formula (IX):



(IX)

or a salt thereof. An exemplary compound of formula (IX) is:



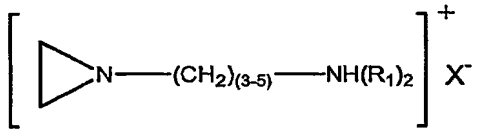
(14)

As mentioned above, in some embodiments, the aziridino ring of the compounds of the invention can be substituted. The aziridino ring of the compounds of the invention can be substitute with a structure X-CH_2-CH_2-N- , wherein X is -Cl, -Br, -F, -I, -O-S(=O)$_2$-CH_3, -O-S(=O)$_2$-CH_2-C_6H_5, or -O-S(=O)$_2$-C_6H_4-CH_3. For example, substituted forms of compounds of formula (V) have the following formula (X):

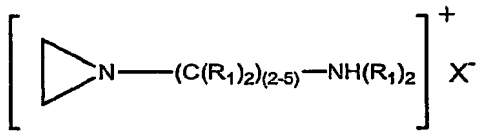
X-CH_2-CH_2-N-$(CH_2)_{(3-5)}$-N$(R_1)_2$ (X)

wherein X is -Cl, -Br, -F, -I, -O-S(=O)$_2$-CH$_3$, -O-S(=O)$_2$-CH$_2$-C$_6$H$_5$, or -O-S(=O)$_2$-C$_6$H$_4$-CH$_3$, each R_1 is, independently, selected from the group consisting of H, C_{2-4} alkenyl, phenyl, and benzyl.

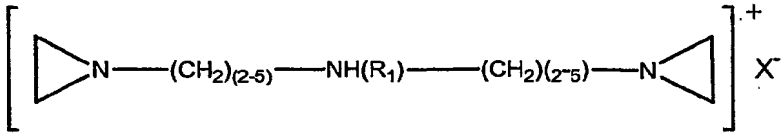
The aziridino compounds of the present invention are protonated (i.e., positively charged) on one or more nitrogen at physiological pH. Thus, in certain embodiments the aziridino compounds used in accordance with the invention are protonated and can be formed as salts with one or more counter ions, preferable pharmaceutically acceptable ions. For example, protonated compounds of formula (V) (VI), and (VII) have the following respective formulas:



(XI)



(XII)



(XIII)

wherein each R_1 is, independently, selected from the group consisting of H, C_{2-4} alkenyl, phenyl, and benzyl, and X is a pharmaceutically acceptable counter-ion (e.g., sulfate, nitrate, halide, tosylate, phosphate, and the like). For compounds within formula (XII) or (XIII), R_1 can also be C_{1-4} alkyl. Compounds falling within formula (XII) also have at least one R_1 that is phenyl or benzyl.

These protonated forms of the compounds, described herein, (also referred to as "salts"), preferably including a pharmaceutically acceptable counter-ion (e.g., sulfate, nitrate, halide, tosylate, phosphate) and their use in the methods of the invention, are specifically included as being part of the invention.

The compounds useful in the invention described herein also include isomers such as diastereomers and enantiomers, mixtures of isomers, including racemic mixtures, solvates, and polymorphs thereof.

In particularly preferred embodiments of the foregoing methods, the aziridino compound is an ethyleneimine oligomer, particularly an ethyleneimine dimer, trimer, or tetramer. In other preferred embodiments, the aziridino compound is not an open ring halogenated, substituted aziridino compound, i.e., having a structure of formula (X), where X is a halogen. In still other preferred embodiments, the aziridino compound includes two or more nitrogen molecules, i.e., not a compound of formula (VIII). In still other preferred embodiments, the aziridino compound does not comprise a benzyl or phenyl ring, i.e. not a compound of formula (VI).

In certain embodiments of the methods for treating infectious disease, the methods further include administering to the subject an amount of one or more non-aziridino antimicrobial compounds effective to treat the infectious disease. The methods are applicable, in preferred embodiments, to infectious diseases including viral infections, bacterial infections, parasite infections and fungal infections.

In certain embodiments of the methods for treating neoplastic disease, the methods further include administering to the subject an amount of one or more non-aziridino antineoplastic compounds effective to treat the neoplastic disease.

In certain embodiments of the methods for treating disorders of immune system function, the methods further include administering to the subject an amount of one or more non-aziridino immune system modulating compounds effective to treat the disorder of immune system function. The methods are applicable, in preferred embodiments, to a subject that has or is suspected of having a leukocyte mediated disease, including autoimmune diseases, graft versus host diseases, allergy, T cell mediated diseases, and B cell mediated diseases. Autoimmune diseases include multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, polymyositis, insulin dependent diabetes, primary biliary cirrhosis, systemic lupus erythematosus, psoriasis, autoimmune hemolytic anemia, mixed connective tissue disease, autoimmune thrombocytopenic purpura and scleroderma.

The methods can be carried out using a variety of routes of administration for the aziridino compounds. In some embodiments, the aziridino compound is administered orally to the subject, preferably formulated with an enteric coating. In other embodiments, the aziridino compound is administered parenterally to the subject, preferably by interarterial, oral, subcutaneous, interperitoneal, intrathecal, intravesicular or intravenous injection. In still other embodiments, the aziridino compound is administered to the subject by implantation of a sustained release formulation.

Administration of the aziridino compounds is performed to deliver an effective amount of the compounds to the subject. Thus, in some embodiments, the aziridino compound is administered to the subject in an amount effective to achieve a peak blood concentrations of at least about 0.1 μg/ml. Preferably the administration results in a peak blood concentration of the aziridino compound from about 1 μg/ml to about 500 μg/ml.

In another aspect of the invention, pharmaceutical compositions are provided. The pharmaceutical compositions include a substantially pure preparation of an aziridino compound as described herein, particularly those described above, and a pharmaceutically acceptable carrier. Preferably the aziridino compound is not an aziridinylquinone compound. In other embodiments, the aziridino compound is not an open ring halogenated, substituted aziridino compound, i.e., having a structure of formula (X), where X is a halogen. In still other embodiments, the aziridino compound includes two or more nitrogen molecules, i.e., not a compound of formula (VIII). In still other embodiments, the aziridino compound does not comprise a benzyl or phenyl ring, i.e. not a compound of formula (VI).

In particularly preferred embodiments of the foregoing pharmaceutical compositions, the aziridino compound is an ethyleneimine oligomer, particularly an ethyleneimine dimer or an ethyleneimine trimer or ethyleneimine tetramer.

The pharmaceutical compositions can be formulated for various routes of delivery, including, in certaim embodiments, oral delivery or parenteral delivery.

In still other embodiments, the pharmaceutical compositions also include one or more non-aziridino antimicrobial compounds, one or more non-aziridino antineoplastic compounds, or one or more non-aziridino immune system modulating compounds.

In a further aspect of the invention, methods for manufacturing a pharmaceutical composition are provided. The methods include placing a substantially pure preparation of an aziridino compound, wherein the aziridino compound is not an aziridinylquinone compound, in a pharmaceutically acceptable carrier.

In some embodiments, the pharmaceutically acceptable carrier is suitable for oral administration. In these embodiments, the methods also can include a step of formulating the composition into a tablet or capsule; preferably the tablet or capsule includes an enteric coating.

In other embodiments, the pharmaceutically acceptable carrier is suitable for parenteral administration. In these embodiments, the methods also can include a step of lyophilizing the composition to form a lyophilized preparation.

Each of the limitations of the invention can encompass various embodiments of the invention. It is, therefore, anticipated that each of the limitations of the invention involving any one element or combinations of elements can be included in each aspect of the invention.

Detailed Description of the Invention

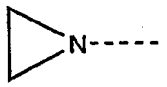
The invention relates to methods and products for the treatment of infectious disease, neoplastic disease and disorders of immune system function using one or more aziridino oligomers. It has now been discovered that aziridino compounds, including ethyleneimine oligomer compounds, can be used *in vivo* for the treatment of the disorders. Due to the nucleic acid modification properties of these compounds, which have been shown to inactivate viruses and other infectious disease agents, the use of these compounds *in vivo* previously was believed to be associated with excessive toxicity in that host nucleic acids would be damaged in addition to the nucleic acid molecules of the infectious agents. In addition, aziridino compounds unexpectedly are selectively toxic for neoplastic cells but not for non-neoplastic cells.

The methods and products for the treatment of infectious disease, neoplastic disease and disorders of immune system function are based on compounds utilizing aziridino chemistry. The aziridino compounds include in certain embodiments aziridino compounds with an alkyl chain, such as ethyleneimine oligomers, which are positively charged electrophilic molecules chemically related to binary ethyleneimine that has selective reactivity with nucleic acids. As used herein, an "ethyleneimine oligomer" can refer to an ethyleneimine dimer, an ethyleneimine trimer, an ethyleneimine tetramer or derivative thereof. Methods for synthesis of aziridino compounds, particularly ethyleneimine oligomers, are provided, for example, in U.S. Patent 6,215,003 and Kostyanovskii et al., "Oligomer of Aziridines and N-β-Aziridinoethylamides," Institute of Chemical Physics of the Academy of Sciences of the U.S.S.R. Moscow. Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya 11:2566-2575 (1988).

The aziridino compounds have a method of action that includes disruption of nucleic acid replication and/or transcription to achieve desirable biological effects. The electrostatic binding of positively charged aziridino compounds such as ethyleneimine oligomers to nucleic acid molecules results in a covalent interaction of the aziridino group with nucleophilic groups of DNA or RNA, predominantly the N-7 position of guanine. Covalent modification of nucleic acid bases can cause loss of the base, i.e., formation of abasic sites, or

even strand breaks. Abasic sites and strand breaks produced by ethyleneimine oligomer adducts with nucleic acids act as potent stop signals for nucleic acid polymerases. Accordingly, the modified nucleic acids can not serve as templates for replication or transcription. As a result, the aziridino compounds are useful for treating *in vivo* a variety of diseases that include those caused by infectious organisms, neoplastic cell growth and inappropriate presence or activity of the immune system cells (e.g., lymphocytes).

Aziridino-containing compounds useful in the methods of the invention preferably contain a moiety having the formula (I):

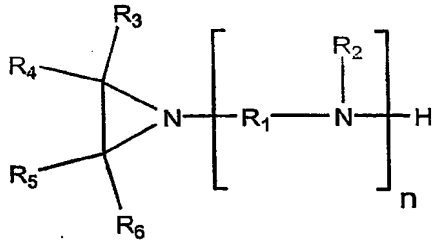


(I)

In this three-membered ring, the two carbons are preferably unsubstituted (i.e., they contain hydrogens), but they can be substituted with aliphatic or aromatic hydrocarbon moieties, each containing between one and four carbon atoms, inclusive.

Various aziridino compounds are disclosed in U.S. Patent 6,093,564, and in U.S. application number 60/378,184, filed on May 6, 2002, entitled Methods and Compositions for the Modification of Nucleic Acids, the entire disclosures of which are incorporated by reference. The therapeutic use of these compounds is provided herein.

In one set of embodiments, the aziridino-containing compound has the formula (II):

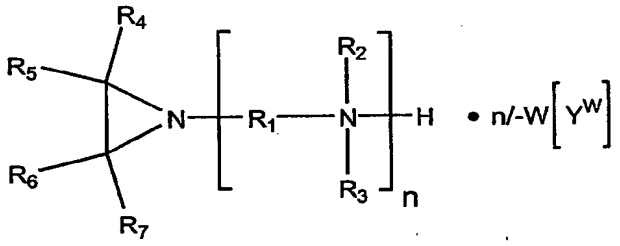


(II)

wherein each R_1 is a divalent hydrocarbon moiety containing between two and four carbon atoms, inclusive; each of R_2, R_3, R_4, R_5, and R_6 is, independently, H or a monovalent hydrocarbon moiety containing between one and four carbon atoms, inclusive; and n is an integer between one and ten, inclusive.

In various preferred embodiments, each R_1 contains two or three carbon atoms; each of R_2, R_3, R_4, R_5, and R_6 is H; and n is one or two. For example, ethyleneimine tetramer fits formula (II) when R_1 contains two carbon atoms, and each of R_2, R_3, R_4, R_5, and R_6 is H, and n is three. Similarly, ethylene trimer fits formula (II) where R_1 contains two carbon atoms, each of R_2, R_3, R_4, R_5, and R_6 is H, and n is two, and ethylene dimer fits formula (II) when R_1 contains two carbon atoms, and each of R_2, R_3, R_4, R_5, and R_6 is H, and n is one.

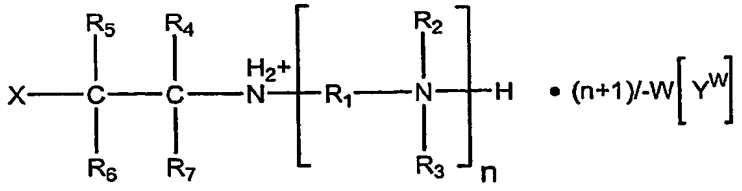
In another set of examples, the compound has the formula (III):



(III)

wherein each R_1 is a divalent hydrocarbon moiety containing between two and four carbon atoms, inclusive; each of R_2, R_3, R_4, R_5, R_6, and R_7 is, independently, H or a monovalent hydrocarbon moiety containing between one and four carbon atoms, inclusive; Y is pharmaceutically acceptable counter anion; W is the valency of Y; and n is an integer between one and ten, inclusive.

Aziridino-containing compounds also include open-ring counterparts to the compounds of formula (I). In one example, aziridino-containing compounds useful in the methods of the invention have the formula (IV):



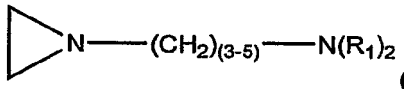
(IV)

wherein each R_1 is a divalent hydrocarbon moiety containing between two and four carbon atoms, inclusive; each of R_2, R_3, R_4, R_5, R_6, and R_7 is, independently, H or a monovalent hydrocarbon moiety containing between one and four carbon atoms, inclusive; X is Cl or Br;

Y is a pharmaceutically acceptable counter anion; W is the valency of Y; and n is an integer between one and ten, inclusive.

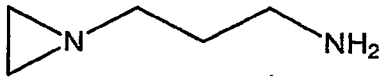
In various preferred embodiments of compounds satisfying formula (III) or formula (IV), each R_1 contains two or three carbon atoms; each of R_2, R_3, R_4, R_5, and R_6 is H; and n is one or two. Suitable counter anions include nitrate, sulfate, halide, phosphate, and tosylate ions.

In an additional set of embodiments, the aziridino-containing compound has the formula (V):

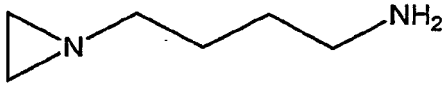


(V)

or a salt thereof, wherein each R_1 is, independently, selected from the group consisting of H, C_{1-4} alkyl, C_{2-4} alkenyl, phenyl, and benzyl. In particular embodiments, the compound is 1-aziridinepropanamine or 1-aziridinebutanamine (compounds 1 and 2, respectively):

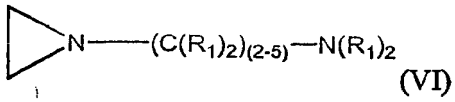


(1)



(2).

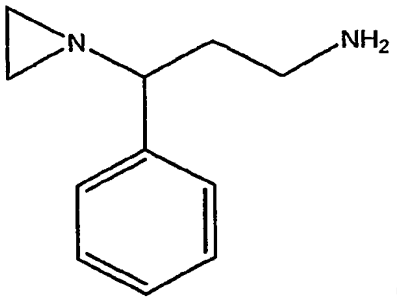
In another additional set of embodiments, the aziridino-containing compound has the formula (VI):



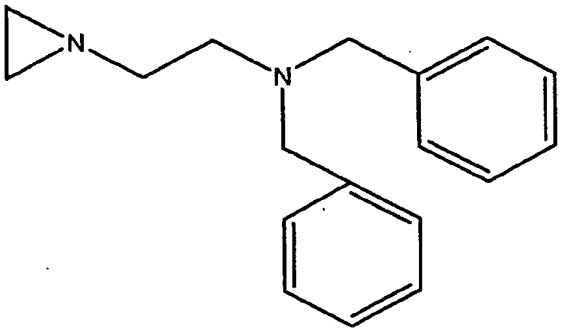
(VI)

or a salt thereof, wherein each R_1 is, independently, selected from the group consisting of H, C_{1-4} alkyl, C_{2-4} alkenyl, phenyl, and benzyl, provided that at least one R_1 is phenyl or benzyl.

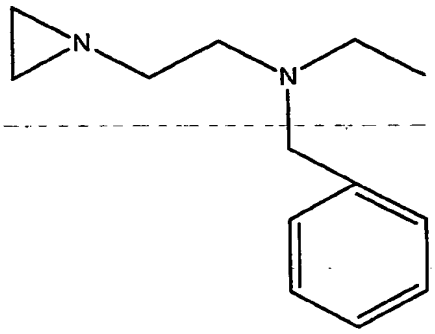
Exemplary aziridino-containing compounds that fall within formula (VI) are 3-phenyl-1-aziridinepropanamine, N,N-dibenzyl-1-aziridineethanamine, and N-benzyl-N-ethyl-1-aziridineethanamine, and 2-benzyl-1-aziridineethanamine (compounds 3, 4, 5, and 6, respectively).



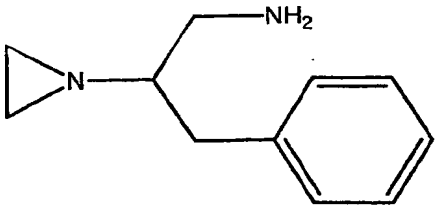
(3)



(4)

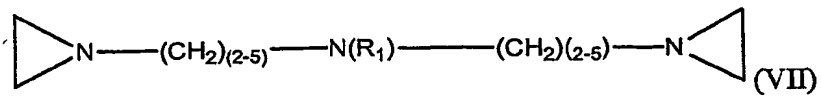


(5)



(6)

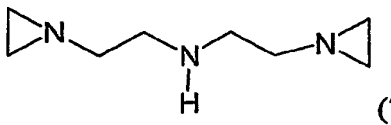
In a further set of embodiments, the aziridino-containing compound has the formula (VII):



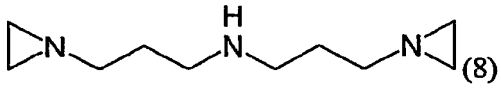
(VII)

or a salt thereof, wherein R_1 is selected from the group consisting of H, C_{1-4} alkyl, C_{2-4} alkenyl, phenyl, and benzyl.

Exemplary compounds that satisfy formula (VII) are 1,1'-[iminobis(dimethylene)]bis aziridine and 1,1'-[iminobis(trimethylene)]bis aziridine (compounds 7 and 8 respectively).

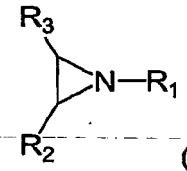


(7)



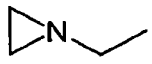
(8)

In an additional set of embodiments, the aziridino-containing compound has the formula:



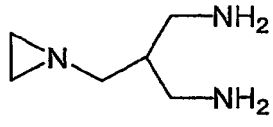
(VIII)

or a salt thereof, wherein R_1 is a C_{1-4} alkyl and R_2 and R_3 is each, independently, H or a C_{1-4} alkyl. An exemplary compound of formula (VIII) is:

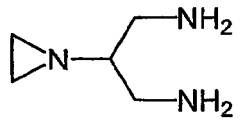


(9)

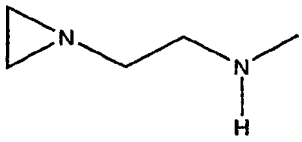
In other embodiments, the aziridino-containing compound is one of the following compounds:



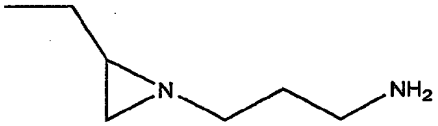
(10)



(11)



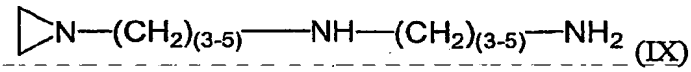
(12)



(13)

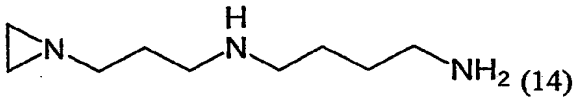
or a salt thereof.

In still another set of embodiments, the aziridino-containing compound has the formula (IX):



(IX)

or a salt thereof. An exemplary compound of formula (IX) is:



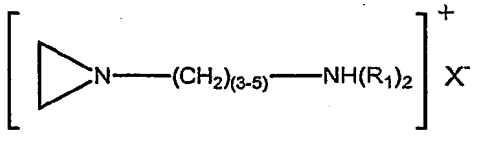
(14)

The aziridino ring of the compounds of the invention can be substituted with a structure X-CH_2-CH_2-N-, wherein X is -Cl, -Br, -F, -I, -O-S(=O)$_2$-CH_3, -O-S(=O)$_2$-CH_2-C_6H_5, or -O-S(=O)$_2$-C_6H_4-CH_3. For example, the substituted forms of compounds of formula (V) have the following formula (X):

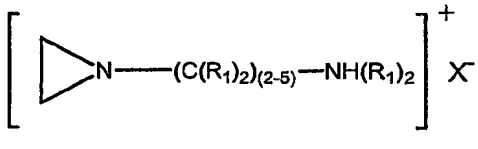
X-CH_2-CH_2-N-$(CH_2)_{(3-5)}$-N$(R_1)_2$ (X)

wherein X is -Cl, -Br, -F, -I, -O-S(=O)$_2$-CH_3, -O-S(=O)$_2$-CH_2-C_6H_5, or -O-S(=O)$_2$-C_6H_4-CH_3, each R_1 is, independently, selected from the group consisting of H, C_{2-4} alkenyl, phenyl, and benzyl.

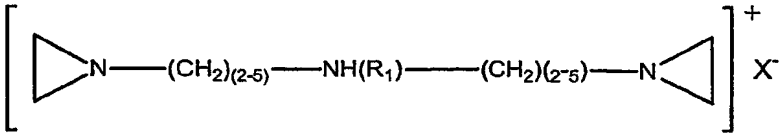
The aziridino compounds of the present invention are protonated (i.e., positively charged) on one or more nitrogen at physiological pH. For example, protonated compounds of formula (V) (VI), and (VII) have the following respective formulas:



(XI)



(XII)



(XIII)

wherein each R_1 is, independently, selected from the group consisting of H, C_{2-4} alkenyl, phenyl, and benzyl, and X is a pharmaceutically acceptable counter-ion (e.g., sulfate, nitrate, halide, tosylate, phosphate, and the like). For compounds within formula (XII) or (XIII), R_1 can also be C_{1-4} alkyl. Compounds falling within formula (XII) also have at least one R_1 that is phenyl or benzyl.

These protonated forms of the compounds, described herein, (also referred to as "salts"), and their use in the methods of the invention, are specifically included as being part of the invention.

The compounds useful in the invention described herein also include isomers such as diastereomers and enantiomers, mixtures of isomers, including racemic mixtures, solvates, and polymorphs thereof.

As used herein, the term "prevent", "prevented", or "preventing" and "treat", "treated" or "treating" when used with respect to the prevention or treatment of an infectious disease refers to a prophylactic treatment which increases the resistance of a subject to a microorganism or, in other words, decreases the likelihood that the subject will develop an infectious disease to the microorganism, as well as to a treatment after the subject has been infected in order to fight the infectious disease, e.g., reduce or eliminate it altogether or prevent it from becoming worse.

As used herein, the term "prevent", "prevented", or "preventing" and "treat", "treated" or "treating" when used with respect to the prevention or treatment of an neoplastic disease refers to prophylatic or therapeutic use of aziridino compounds to reduce or eliminate neoplastic disease from a subject. Although not wishing to be bound by theory, it is believed

that the aziridino compounds described herein modify the nucleic acids of the neoplastic cells to render the cells nonproliferative, and/or induce an apoptotic response in the neoplastic cells.

As used herein, the term "prevent", "prevented", or "preventing" and "treat", "treated" or "treating" when used with respect to the prevention or treatment of disorders of immune system function refers to prophylatic or therapeutic use of aziridino compounds to reduce or eliminate immune system disease from a subject. Although not wishing to be bound by theory, it is believed that the aziridino compounds described herein modify the nucleic acids of the immune system cells (e.g., T cells, B cells) that cause the immune system disorder to render the cells nonproliferative, and/or induce an apoptotic response in the immune system cells.

Thus the aziridino compounds are useful for treating or preventing infectious diseases, neoplastic diseases and disorders of immune system function in a subject. In some embodiments, one or more aziridino compounds are used alone (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more aziridino compounds) in the therapeutic methods of the invention. In other embodiments, one or more aziridino compounds are used in combination with other therapeutic compounds in the methods of the invention (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more aziridino compounds in combination with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more non-aziridino compounds).

As used herein, a "subject" shall mean a human, a vertebrate mammal including but not limited to a dog, cat, horse, cow, pig, sheep, goat, or non-human primate, e.g., monkey, or a fowl, e.g., chicken. Included within the scope of the present invention are all animals which are susceptible to infectious diseases, neoplastic diseases and disorders of immune system function.

The aziridino compounds are useful in some aspects of the invention as a prophylactic for the treatment of a subject at risk of developing an infectious disease, neoplastic disease or disorder of immune system function. For example, a subject at risk of infectious disease is one for whom the exposure to a microorganism or expected exposure to a microorganism is known or suspected. A "subject at risk" of developing an infectious disease as used herein is a subject who has any risk of exposure to a microorganism, e.g. someone who is in contact with an infected subject or who is travelling to a place where a particular microorganism is found. For instance, a subject at risk may be a subject who is planning to travel to an area where a particular microorganism is found or it may even be any subject living in an area

where a microorganism has been identified. A subject at risk of developing an infectious disease includes those subjects that have a general risk of exposure to a microorganism, e.g., influenza, but that don't have the active disease during the treatment of the invention as well as subjects that are considered to be at specific risk of developing an infectious disease because of medical or environmental factors, that expose them to a particular microorganism. Likewise, a subject at risk of having neoplastic disease or a disorder of immune system function is one who has a medical history of neoplastic disease or a disorder of immune system function (including a family history).

The aziridino compounds also are useful in other aspects of the invention as a therapeutic for the treatment of a subject that has or is suspected of having an infectious disease, neoplastic disease or an immunological disorder. A "subject having or suspected of having an infectious disease" is a subject that has had contact with a microorganism. Thus the microorganism has invaded the body of the subject. The word "invade" as used herein refers to contact by the microorganism with the external surface of the subject, e.g., skin or mucosal membranes and/or refers to the penetration of the external surface of the subject by the microorganism. A "subject having or suspected of having neoplastic disease or a disorder of immune system function" is one who is diagnosed with neoplastic disease or a disorder of immune system function, who has neoplastic disease or a disorder of immune system function, or who had neoplastic disease or a disorder of immune system function (e.g., is in remission).

In addition to the use of the aziridino compounds for therapeutic or prophylactic treatments, the invention also encompasses the use of a combination of drugs for the treatment of a subject having an infectious disease, neoplastic disease or a disorder of immune system function, as detailed further herein. In particular, one or more aziridino compounds can be combined with one or more non-aziridino compounds effective to treat one or more of the disorders identified herein.

An "infectious disease" as used herein, refers to a disorder arising from the invasion of a host, superficially, locally, or systemically, by an infectious microorganism. Infectious microorganisms include bacteria, viruses, parasites and fungi.

Bacteria are unicellular organisms which multiply asexually by binary fission. They are classified and named based on their morphology, staining reactions, nutrition and metabolic requirements, antigenic structure, chemical composition, and genetic homology. Bacteria can be classified into three groups based on their morphological forms, spherical

(coccus), straight-rod (bacillus) and curved or spiral rod (vibrio, campylobacter, spirillum, and spirochaete). Bacteria are also more commonly characterized based on their staining reactions into two classes of organisms, gram-positive and gram-negative. Gram refers to the method of staining which is commonly performed in microbiology labs. Gram-positive organisms retain the stain following the staining procedure and appear a deep violet color. Gram-negative organisms do not retain the stain but take up the counter-stain and thus appear pink.

Bacteria have two main structural components, a rigid cell wall and protoplast (material enclosed by the cell wall). The protoplast includes cytoplasm and genetic material. Surrounding the protoplast is the cytoplasmic membrane which includes some of the cell respiratory enzymes and is responsible for the permeability of bacteria and transport of many small molecular weight substances. The cell wall surrounding the cytoplasmic membrane and protoplast is composed of mucopeptides which include complex polymers of sugars cross-linked by peptide chains of amino acids. The wall is also composed of polysaccharides and teichoic acids.

Infectious bacteria include, but are not limited to, gram negative and gram positive bacteria. Gram positive bacteria include, but are not limited to *Pasteurella* species, *Staphylococci* species, and *Streptococcus* species. Gram negative bacteria include, but are not limited to, *Escherichia coli*, *Pseudomonas* species, and *Salmonella* species. Specific examples of infectious bacteria include but are not limited to: *Helicobacter pyloris*, *Borelia burgdorferi*, *Legionella pneumophilia*, *Mycobacteria* species (e.g. *M. tuberculosis*, *M. avium*, *M. intracellulare*, *M. kansaii*, *M. gordonae*), *Staphylococcus aureus*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Listeria monocytogenes*, *Streptococcus pyogenes* (Group A Streptococcus), *Streptococcus agalactiae* (Group B Streptococcus), *Streptococcus* (viridans group), *Streptococcus faecalis*, *Streptococcus bovis*, *Streptococcus* (anaerobic species), *Streptococcus pneumoniae*, pathogenic *Campylobacter* species, *Enterococcus* species, *Haemophilus influenzae*, *Bacillus antracis*, *Corynebacterium diphtheriae*, *Erysipelothrix rhusiopathiae*, *Clostridium perfringers*, *Clostridium tetani*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pasturella multocida*, *Bacteroides* species, *Fusobacterium nucleatum*, *Streptobacillus moniliformis*, *Treponema pallidium*, *Treponema pertenue*, *Leptospira* species, *Rickettsia* species, and *Actinomyces israelli*. Additional exemplary bacteria are *Mycoplasma, e.g. Mycoplasma pneumoniae*, *Chlamydophila*, e.g. *Chlamydophila pneumoniae*, *Bartonella* species, and *Tropheryma whippelii*.

Viruses are small infectious agents which contain a nucleic acid core and a protein coat, but are not independently living organisms. A virus cannot survive in the absence of a living cell within which it can replicate. Viruses enter specific living cells either by endocytosis or direct injection of DNA (phage) and multiply, causing disease. The multiplied virus can then be released and infect additional cells. Some viruses are DNA-containing viruses and other are RNA-containing viruses.

Once the virus enters the cell it can cause a variety of physiological effects. One effect is cell degeneration, in which the accumulation of virus within the cell causes the cell to die and break into pieces and release the virus. Another effect is cell fusion, in which infected cells fuse with neighboring cells to produce syncytia. Other types of virus cause cell proliferation which results in tumor formation.

Specific examples of viruses that have been found in humans include but are not limited to: *Retroviridae* (e.g. human immunodeficiency viruses, such as HIV-1 (also referred to as HTLV-III, LAV or HTLV-III/LAV, or HIV-III); and other isolates, such as HIV-LP); *Picornaviridae* (e.g. polio viruses, hepatitis A virus; enteroviruses, human Coxsackie viruses, rhinoviruses, echoviruses); *Calciviridae* (e.g. strains that cause gastroenteritis); *Togaviridae* (e.g. equine encephalitis viruses, rubella viruses); *Flaviridae* (e.g. dengue viruses, encephalitis viruses, yellow fever viruses); *Coronoviridae* (e.g. coronaviruses); *Rhabdoviradae* (e.g. vesicular stomatitis viruses, rabies viruses); *Filoviridae* (e.g. ebola viruses); *Paramyxoviridae* (e.g. parainfluenza viruses, mumps virus, measles virus, respiratory syncytial virus); *Orthomyxoviridae* (e.g. influenza viruses); *Bunyaviridae* (e.g. Hantaan viruses, bunyaviruses, phleboviruses and Nairo viruses); *Arenaviridae* (hemorrhagic fever viruses); *Reoviridae* (e.g. reoviruses, orbiviurses and rotaviruses); *Birnaviridae*; *Hepadnaviridae* (Hepatitis B virus); *Parvovirida* (parvoviruses); *Papovaviridae* (papilloma viruses, polyoma viruses); *Adenoviridae* (most adenoviruses); *Herpesviridae* (herpes simplex virus (HSV) 1 and 2, varicella zoster virus, cytomegalovirus (CMV), herpes virus); *Poxviridae* (variola viruses, vaccinia viruses, pox viruses); and *Iridoviridae* (e.g. African swine fever virus); and unclassified viruses (e.g., the agent of delta hepatitis (thought to be a defective satellite of hepatitis B virus), the agents of non-A, non-B hepatitis (class 1 = internally transmitted; class 2 = parenterally transmitted (i.e. Hepatitis C); Norwalk and related viruses, and astroviruses).

In addition to viruses that infect human subjects causing human disorders, the invention is also useful for treating other non-human vertebrates. Non-human vertebrates are

also capable of developing infections which can be prevented or treated with the combinations of aziridino compounds and anti-microbials disclosed herein. For instance, in addition to the treatment of infectious human diseases, the methods of the invention are useful for treating or preventing infections of non-human animals.

Infectious virus of both human and non-human vertebrates, include retroviruses, RNA viruses and DNA viruses. This group of retroviruses includes both simple retroviruses and complex retroviruses. The simple retroviruses include the subgroups of B-type retroviruses, C-type retroviruses and D-type retroviruses. An example of a B-type retrovirus is mouse mammary tumor virus (MMTV). The C-type retroviruses include subgroups C-type group A (including Rous sarcoma virus (RSV), avian leukemia virus (ALV), and avian myeloblastosis virus (AMV)) and C-type group B (including murine leukemia virus (MLV), feline leukemia virus (FeLV), murine sarcoma virus (MSV), gibbon ape leukemia virus (GALV), spleen necrosis virus (SNV), reticuloendotheliosis virus (RV) and simian sarcoma virus (SSV)). The D-type retroviruses include Mason-Pfizer monkey virus (MPMV) and simian retrovirus type 1 (SRV-1). The complex retroviruses include the subgroups of lentiviruses, T-cell leukemia viruses and the foamy viruses. Lentiviruses include HIV-1, but also include HIV-2, SIV, Visna virus, feline immunodeficiency virus (FIV), and equine infectious anemia virus (EIAV). The T-cell leukemia viruses include HTLV-1, HTLV-II, simian T-cell leukemia virus (STLV), and bovine leukemia virus (BLV). The foamy viruses include human foamy virus (HFV), simian foamy virus (SFV) and bovine foamy virus (BFV).

Examples of other RNA viruses that are pathogens in vertebrate animals include, but are not limited to, the following: members of the family *Reoviridae*, including the genus Orthoreovirus (multiple serotypes of both mammalian and avian retroviruses), the genus Orbivirus (Bluetongue virus, Eugenangee virus, Kemerovo virus, African horse sickness virus, and Colorado Tick Fever virus), the genus Rotavirus (human rotavirus, Nebraska calf diarrhea virus, murine rotavirus, simian rotavirus, bovine or ovine rotavirus, avian rotavirus); the family *Picornaviridae*, including the genus Enterovirus (poliovirus, Coxsackie virus A and B, enteric cytopathic human orphan (ECHO) viruses, hepatitis A virus, Simian enteroviruses, Murine encephalomyelitis (ME) viruses, Poliovirus muris, Bovine enteroviruses, Porcine enteroviruses , the genus Cardiovirus (Encephalomyocarditis virus (EMC), Mengovirus), the genus Rhinovirus (Human rhinoviruses including at least 113 subtypes; other rhinoviruses), the genus Apthovirus (Foot and Mouth disease (FMDV); the family *Calciviridae*, including Vesicular exanthema of swine virus, San Miguel sea lion

virus, Feline picornavirus and Norwalk virus; the family *Togaviridae*, including the genus Alphavirus (Eastern equine encephalitis virus, Semliki forest virus, Sindbis virus, Chikungunya virus, O'Nyong-Nyong virus, Ross river virus, Venezuelan equine encephalitis virus, Western equine encephalitis virus), the genus Flavirus (Mosquito borne yellow fever virus, Dengue virus, Japanese encephalitis virus, St. Louis encephalitis virus, Murray Valley encephalitis virus, West Nile virus, Kunjin virus, Central European tick borne virus, Far Eastern tick borne virus, Kyasanur forest virus, Louping III virus, Powassan virus, Omsk hemorrhagic fever virus), the genus Rubivirus (Rubella virus), the genus Pestivirus (Mucosal disease virus, Hog cholera virus, Border disease virus); the family *Bunyaviridae*, including the genus Bunyavirus (Bunyamwera and related viruses, California encephalitis group viruses), the genus Phlebovirus (Sandfly fever Sicilian virus, Rift Valley fever virus), the genus Nairovirus (Crimean-Congo hemorrhagic fever virus, Nairobi sheep disease virus), and the genus Uukuvirus (Uukuniemi and related viruses); the family *Orthomyxoviridae*, including the genus influenza virus (influenza virus type A, many human subtypes); Swine influenza virus, and Avian and Equine Influenza viruses; influenza type B (many human subtypes), and influenza type C (possible separate genus); the family *Paramyxoviridae*, including the genus Paramyxovirus (Parainfluenza virus type 1, Sendai virus, Hemadsorption virus, Parainfluenza viruses types 2 to 5, Newcastle Disease Virus, Mumps virus), the genus Morbillivirus (Measles virus, subacute sclerosing panencephalitis virus, distemper virus, Rinderpest virus), the genus Pneumovirus (respiratory syncytial virus (RSV), Bovine respiratory syncytial virus and Pneumonia virus of mice); the family *Rhabdoviridae*, including the genus Vesiculovirus (VSV), Chandipura virus, Flanders-Hart Park virus), the genus Lyssavirus (Rabies virus), fish Rhabdoviruses, and two probable Rhabdoviruses (Marburg virus and Ebola virus); the family *Arenaviridae*, including Lymphocytic choriomeningitis virus (LCM), Tacaribe virus complex, and Lassa virus; the family *Coronoaviridae*, including Infectious Bronchitis Virus (IBV), Mouse Hepatitis virus, Human enteric corona virus, and Feline infectious peritonitis (Feline coronavirus).

Illustrative DNA viruses that infect vertebrate animals include, but are not limited to: the family *Poxviridae*, including the genus Orthopoxvirus (Variola major, Variola minor, Monkeypox ,Vaccinia, Cowpox, Buffalopox, Rabbitpox, Ectromelia), the genus Leporipoxvirus (Myxoma, Fibroma), the genus Avipoxvirus (Fowlpox, other avian poxvirus), the genus Capripoxvirus (sheeppox, goatpox), the genus Suipoxvirus (Swinepox), the genus Parapoxvirus (contagious postular dermatitis virus, pseudocowpox, bovine papular stomatitis

virus); the family *Iridoviridae* (African swine fever virus, Frog viruses 2 and 3, Lymphocystis virus of fish); the family *Herpesviridae*, including the alpha-Herpesviruses (Herpes Simplex Types 1 and 2, Varicella-Zoster, Equine abortion virus, Equine herpes virus 2 and 3, pseudorabies virus, infectious bovine keratoconjunctivitis virus, infectious bovine rhinotracheitis virus, feline rhinotracheitis virus, infectious laryngotracheitis virus) the Beta-herpesviruses (Human cytomegalovirus and cytomegaloviruses of swine, monkeys and rodents); the gamma-herpesviruses (Epstein-Barr virus (EBV), Marek's disease virus, Herpes saimiri, Herpesvirus ateles, Herpesvirus sylvilagus, guinea pig herpes virus, Lucke tumor virus); the family *Adenoviridae*, including the genus Mastadenovirus (Human subgroups A,B,C,D,E and ungrouped); simian adenoviruses (at least 23 serotypes), infectious canine hepatitis, and adenoviruses of cattle, pigs, sheep, frogs and many other species, the genus Aviadenovirus (Avian adenoviruses); and non-cultivatable adenoviruses; the family *Papoviridae*, including the genus Papillomavirus (Human papilloma viruses, bovine papilloma viruses, Shope rabbit papilloma virus, and various pathogenic papilloma viruses of other species), the genus Polyomavirus (polyomavirus, Simian vacuolating agent (SV-40), Rabbit vacuolating agent (RKV), K virus, BK virus, JC virus, and other primate polyoma viruses such as Lymphotrophic papilloma virus); the family *Parvoviridae* including the genus Adeno-associated viruses, and the genus Parvovirus (Feline panleukopenia virus, bovine parvovirus, canine parvovirus, Aleutian mink disease virus, etc).

Parasites are organisms which depend upon other organisms in order to survive and thus must enter, or infect, another organism to continue their life cycle. The infected organism, i.e., the host, provides both nutrition and habitat to the parasite. The term "parasite" as used herein refers to protozoa, helminths, and ectoparasitic arthropods (e.g., ticks, mites, etc.). Protozoa are single celled organisms which can replicate both intracellularly and extracellularly, particularly in the blood, intestinal tract or the extracellular matrix of tissues. Helminths are multicellular organisms which almost always are extracellular (the exception being *Trichinella*). Helminths normally require exit from a primary host and transmission into a secondary host in order to replicate. In contrast to these aforementioned classes, ectoparasitic arthropods form a parasitic relationship with the external surface of the host body.

Parasites can be classified based on whether they are intracellular or extracellular. An "intracellular parasite" as used herein is a parasite whose entire life cycle is intracellular. Examples of human intracellular parasites include *Leishmania*, *Plasmodium*, *Trypanosoma*

cruzi, *Toxoplasma gondii*, *Babesia*, and *Trichinella spiralis*. An "extracellular parasite" as used herein is a parasite whose entire life cycle is extracellular. Extracellular parasites capable of infecting humans include *Entamoeba histolytica*, *Giardia lamblia*, *Enterocytozoon bieneusi*, *Naegleria* and *Acanthamoeba* as well as most helminths. Yet another class of parasites is defined as being mainly extracellular but with an obligate intracellular existence at a critical stage in their life cycles. Such parasites are referred to herein as "obligate intracellular parasites". These parasites may exist most of their lives or only a small portion of their lives in an extracellular environment, but they all have at lest one obligate intracellular stage in their life cycles. This latter category of parasites includes *Trypanosoma rhodesiense* and *Trypanosoma gambiense*, *Isospora*, *Cryptosporidium*, *Eimeria*, *Neospora*, *Sarcocystis*, and *Schistosoma*. In one aspect, the invention relates to the prevention and treatment of infection resulting from intracellular parasites and obligate intracellular parasites which have at least in one stage of their life cycle that is intracellular. In some embodiments, the invention is directed to the prevention of infection from obligate intracellular parasites which are predominantly intracellular. An exemplary and non-limiting list of parasites for some aspects of the invention is provided herein.

Blood-borne and/or tissues parasites include *Plasmodium*, *Babesia microti*, *Babesia divergens*, *Leishmania tropica*, *Leishmania*, *Leishmania braziliensis*, *Leishmania donovani*, *Trypanosoma gambiense* and *Trypanosoma rhodesiense* (African sleeping sickness), *Trypanosoma cruzi* (Chagas' disease), and *Toxoplasma gondii*.

Typical parasites infecting horses are *Gasterophilus*; *Eimeria leuckarti*, *Giardia*; *Tritrichomonas equi*; *Babesia* (RBCs), *Theileria equi*; *Trypanosoma*; *Klossiella equi*; *Sarcocystis*.

Typical parasites infecting swine include *Eimeria bebliecki*, *Eimeria scabra*, *Isospora suis*, *Giardia*; *Balantidium coli*, *Entamoeba histolytica*; *Toxoplasma gondii* and *Sarcocystis*, and *Trichinella spiralis*.

The major parasites of dairy and beef cattle include *Eimeria*, *Cryptosporidium*, *Giardia*; *Toxoplasma gondii*; *Babesia bovis* (RBCs), *Babesia bigemina* (RBCs), *Trypanosoma* (plasma), *Theileria* (RBC); *Theileria parva* (lymphocytes); *Tritrichomonas foetus*; and *Sarcocystis*.

Typical parasites infecting sheep and goats include *Eimeria*, *Cryptosporidium*, *Giardia*; *Toxoplasma gondii*; *Babesia* (RBC), *Trypanosoma* (plasma), *Theileria* (RBC); and *Sarcocystis*.

Typical parasitic infections in poultry include coccidiosis caused by *Eimeria acervulina, E. necatrix, E. tenella, Isospora* and *Eimeria truncata*; histomoniasis, caused by *Histomonas meleagridis* and *Histomonas gallinarum*; trichomoniasis caused by *Trichomonas gallinae*; and hexamitiasis caused by *Hexamita meleagridis*. Poultry can also be infected *Emeria maxima, Emeria meleagridis, Eimeria adenoeides, Eimeria meleagrimitis, Cryptosporidium, Eimeria brunetti, Emeria adenoeides, Leucocytozoon, Plasmodium, Hemoproteus meleagridis, Toxoplasma gondii* and *Sarcocystis*.

Parasitic infections also pose serious problems in laboratory research settings involving animal colonies. Some examples of laboratory animals intended to be treated, or in which parasite infection is sought to be prevented, by the methods of the invention include mice, rats, rabbits, guinea pigs, nonhuman primates, as well as the aforementioned swine and sheep.

Typical parasites in mice include *Leishmania, Plasmodium berghei, Plasmodium yoelii, Giardia muris, Hexamita muris*; *Toxoplasma gondii; Trypanosoma duttoni* (plasma); *Klossiella muris*; *Sarcocystis*. Typical parasites in rats include *Giardia muris, Hexamita muris*; *Toxoplasma gondii*; *Trypanosoma lewisi* (plasma); *Trichinella spiralis*; and *Sarcocystis*. Typical parasites in rabbits include *Eimeria*; *Toxoplasma gondii*; *Nosema cuniculi*; *Eimeria stiedae*, and *Sarcocystis*. Typical parasites of the hamster include *Trichomonas*; *Toxoplasma gondii*; *Trichinella spiralis*; and *Sarcocystis*. Typical parasites in the guinea pig include *Balantidium caviae*; *Toxoplasma gondii*; *Klossiella caviae;* and *Sarcocystis*.

Fungi are eukaryotic organisms, only a few of which cause infection in vertebrate mammals. Because fungi are eukaryotic organisms, they differ significantly from prokaryotic bacteria in size, structural organization, life cycle and mechanism of multiplication. Fungi are classified generally based on morphological features, modes of reproduction and culture characteristics. Although fungi can cause different types of disease in subjects, such as respiratory allergies following inhalation of fungal antigens, fungal intoxication due to ingestion of toxic substances, such as amatatoxin and phallotoxin produced by poisonous mushrooms and aflotoxins, produced by aspergillus species, not all fungi cause infectious disease.

Infectious fungi can cause systemic or superficial infections. Primary systemic infection can occur in normal healthy subjects and opportunistic infections, are most frequently found in immuno-compromised subjects. The most common fungal agents

causing primary systemic infection include *Blastomyces*, *Coccidioides*, and *Histoplasma*. Common fungi causing opportunistic infection in immuno-compromised or immunosuppressed subjects include, but are not limited to, *Candida albicans* (an organism which is normally part of the respiratory tract flora), *Cryptococcus neoformans* (sometimes in normal flora of respiratory tract), and various *Aspergillus* species. Systemic fungal infections are invasive infections of the internal organs. The organism usually enters the body through the lungs, gastrointestinal tract, or intravenous lines. These types of infections can be caused by primary pathogenic fungi or opportunistic fungi.

Superficial fungal infections involve growth of fungi on an external surface without invasion of internal tissues. Typical superficial fungal infections include cutaneous fungal infections involving skin, hair, or nails. An example of a cutaneous infection is *Tinea* infections, such as ringworm, caused by *Dermatophytes*, such as *Microsporum* or *Traicophyton* species, i.e., *Microsporum canis*, *Microsporum gypsum*, *Tricofitin rubrum*. Examples of fungi include: *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Coccidioides immitis*, *Blastomyces dermatitidis*, *Chlamydia trachomatis*, *Candida albicans*.

Diseases associated with fungal infection include aspergillosis, blastomycosis, camdidiais, chromoblastomycosis, coccidioidomycosis, cryptococcosis, fungal eye infections, fungal hair, nail, and skin infections, histoplasmosis, lobomycosis, mycetoma, otomycosis, paracoccidioidomycosis, penicilliosis, marneffeii, phaeohyphomycosis, rhinosporidioisis, sporotrichosis, and zygomycosis.

Aspergillosis is a disease caused by the fungi of the genus *Aspergillus*, which can lead to mild or severe disease, generally depending on factors such as the status of the host immune system. Aspergillus frequently arises as an opportunistic infection in patients having immune-suppressive diseases, or being treated with chemotherapy. Some forms of aspergillus can be treated with prednisone, disodium chromoglycat, nystatin, amphotericin B, itraconazole, or voriconazole.

Blastomycosis is a fungal infection arising from the organism *Blastomyces dermatitis*. The infection initiates in the lungs and usually is disseminated to other body sites, especially the skin and bone. It is treated by amphotericin B, hydroxystilbamidine, itraconazole and voriconazole. When amphotericin B is used, at least 1.5 grams must be given to avoid relapse. However, when the drug is administered in combination with the aziridino compounds, lower doses can be given without a relapse. Generally hydroxystilbamidine has been used for treating the cutaneous form of the disease but not other forms. When combined

with aziridino compounds in the combination compositions of the invention, it can also be used for the treatment of other forms, as well as in lower doses for the cutaneous form.

Candidiasis is a fungal infection caused by a member of the genus *Candida*. The disease can be in the form of allergic, cutaneous, mucocutaneous, or systemic candidiasis. Nystatin is used for the treatment of the cutaneous, mucocutaneous, and allergic diseases. Amphotericin B is useful for treating this systemic disease. Other drugs useful for the treatment include 5-fluorocytosine, fluconazole, itraconazole and voriconazole.

Chromoblastomycosis is a chronic infection of the skin and subcutaneous tissue. Although the infection is usually localized, parts can disseminate systemically and in particular to the brain. Itraconazole and terbinafine are the drugs used to treat this infection. The principal fungi causing this infection are *Cladophialophora, Carrionii, Fonsecaea, Compacta, Fonsecaea pedrosoi, Phialophora, Verruceosa, Rhinocladiella*, and *Aquasbera*.

Coccidioidomycosis is a fungal disease of the respiratory tract which can be acute, chronic, severe or fatal. The disease is primarily caused by *Coccidioides immitis*. Amphotericin B, itraconazole, fluconazole, ketaconazole, and voriconazole are anti-fungal agents that are used for the treatment of this disorder.

Cryptococcosis is a fungal disorder caused by *Cryptococcus norformans* or *Filobasidiella neoformans*. The disease can take the form of a chronic, subacute, acute, pulmonary, systemic, or meningitic disease, following primary infection in the lungs. If the disease spreads from the lungs to the central nervous system, it is usually treated immediately with amphotericin B and/or 5-fluorocytosine and in some cases fluconazole.

Fungal infections of the eye include mycotic keratitis, and endogenous or extension oculomycosis. Mycotic keratitis is caused by a variety of fungi including *Acremonium, Aspergillus, Bipolaris, Candida albicans, Curvularia, Exserohilum, Fusarium*, and *Lasiodiplodia*. Amphotericin B is not used for treatment because it irritates the infected tissue. Drugs useful for treating mycotic keratitis include pimaricin and fluconazole. Oculomycosis is generally caused by *Candida albicans* or rhizopus, arrhizus. Amphotericin B is the anti-fungal agent used for treatment.

Fungal infections of the hair, nail, and skin include onychomycosis, piedra, pityriasis versicolor, tinea barbae, tinea capitis, tinea corporis, tinea cruris, tinea faosa, tinea nigra, tinea unguium. Onychomycosis, which is generally caused by fungi such as *Acremonium, Aspergillus, Candida, Fusarium, Scopulariopisis, Onychocola*, and *Scytalidium*, can be treated with itraconazole, turbinifine, amphotericin B, gentian violet, resorcin, iodine,

nystatin, thiabendazole, and glutarardehyde. Piedra, which is a colonization of the hair shaft to bifungal organisms such as *Piedraia* and *Trichosporin*, can be treated with keratolytic agents, mild fungicides, fluconazole, and itraconazole. The tineas are various forms of ringworm colonizing different bodily regions. These diseases are generally caused by fungi such as *Microsporum*, *Trichophyton*, and *Epidermophyton*. The tineas can be treated with keratolytic agents, intraconazole, turbinifine, tolnaftate, clotrimazole, miconazole, econazole, and ketaconzole.

Histoplasmosis (capsulati and duboisii) are fungal infections caused by *Histoplasma* and *Ajellomyces*. *Histoplasmosis capsulati* can adequately be treated with amphotericin B, itraconazole or voriconazole. If the subject being treated has AIDS, fluconazole is usually used. *Histoplasmosis duboisii* once it becomes disseminated, especially to the liver or spleen, is very difficult to treat. Amphotericin B, itraconazole, fluconazole, and voriconazole are used. When these compounds are combined with the aziridino compounds of the invention, prognosis is improved.

Lobomycosis is a fungal infection caused by *Lacazia loboi*. Lobomycosis is a cutaneous infection which develops into lesions which can be removed by surgery. There are not drugs specifically used for this disorder. Mycetoma is an infection caused by a variety of fungi including *Eumycotic*, *Acromonium*, *Aspergillus*, *Exophiala*, *Leptos Phaeria*, *Madurella*, *Neotestudina*, *Pseudallesheria*, and *Pyrenochieta*. The disease involves lesions of the cutaneous and subcutaneous tissues, which can rupture and spread to surrounding tissues. The mycetomas can be treated with ketoconazole, in combination with surgery.

Otomycosis is a fungal ear infection caused by *Aspergillus* or *candida*. The infection is a superficial infection of the outer ear canal, which is characterized by inflammation, pruritus, scaling, and sever discomfort. It is a chronic recurring mycosis.

Paracoccidioidomycosis is a fungal infection cause by *Paracoccidioides brasiliensis*. The disease originates as a pulmonary infection and can disseminate into the nasal, buccal, and gastrointestinal mucosa. Amphotericin B and sulfonamides are generally used to treat the disease.

Phaeohyphomycosis is a fungal infection caused by a variety of fungi including *Cladophialophora*, *Curvularia*, *Bipolaris*, *Exserohilum*, *Exophiala*, *Scedosporium*, *Ochroconis*, *Coniothyrium*, *Phialophora*, *Wangiella*, and *Lasiodiplodia*. The infection can be localized or can invade various tissues including the brain, bone, eyes, and skin. Invasion of the brain or bone can be lethal. Generally, phaeohyphomycosis is treated with amphotericin

B and phyfluorocytozine or intaconazole. Rhinosporidiosis is an infection of the mucus membrane caused by *Rhinosporidium seeberi*. Local injection of amphotericin B is used as treatment.

Sporotrichosis is a chronic infection of the cutaneous tissues, subcutaneous tissues, or lymph system. The infection may also spread to tissues such as bone, muscle, CNS, lungs, and/or genitourinary system. Usually the fungi *Sporothrix schenckii* is inhaled or passed through a lesion in the skin. Sporotrichosis is usually treated with oral potassium iodide, amphotericin B, or 5-fluorocytozine.

Zygomycosis is a chronic infection caused by *Conidobolus* and *Basidiobolus ranarum*. The disease is treated by potassium iodide and/or amphotericin B.

Other medically relevant microorganisms and the diseases they cause have been described extensively in the literature, e.g., see C.G.A. Thomas, *Medical Microbiology*, Bailliere Tindall, Great Britain 1983, the entire contents of which is hereby incorporated by reference. Each of the foregoing lists is illustrative, and is not intended to be limiting.

The methods of the invention involve, in some aspects, combinations of aziridino compounds and anti-microbial agents for the treatment or prevention of infectious disease. An anti-microbial agent, as used herein, refers to a naturally-occurring or synthetic compound which is capable of killing or inhibiting infectious microorganisms. The type of anti-microbial agent useful according to the invention will depend upon the type of microorganism with which the subject is infected or at risk of becoming infected. It is contemplated that several different kinds of anti-microbial agents can be combined with the aziridino compounds to make compositions useful for treating multifactorial diseases (e.g., HIV infection with opportunistic fungal infections)

One type of anti-microbial agent is an antibacterial agent. Antibacterial agents kill or inhibit the growth or function of bacteria. A large class of antibacterial agents is antibiotics. Antibiotics, which are effective for killing or inhibiting a wide range of bacteria, are referred to as broad spectrum antibiotics. Other types of antibiotics are predominantly effective against the bacteria of the class gram-positive or gram-negative. These types of antibiotics are referred to as narrow spectrum antibiotics. Other antibiotics which are effective against a single organism or disease and not against other types of bacteria, are referred to as limited spectrum antibiotics.

Antibacterial agents are sometimes classified based on their primary mode of action. In general, antibacterial agents are cell wall synthesis inhibitors, cell membrane inhibitors,

protein synthesis inhibitors, nucleic acid synthesis or functional inhibitors, and competitive inhibitors. Cell wall synthesis inhibitors inhibit a step in the process of cell wall synthesis, and in general in the synthesis of bacterial peptidoglycan. Cell wall synthesis inhibitors include β-lactam antibiotics, natural penicillins, semi-synthetic penicillins, ampicillin, clavulanic acid, cephalolsporins, and bacitracin.

The β-lactams are antibiotics containing a four-membered β-lactam ring which inhibits the last step of peptidoglycan synthesis. β-lactam antibiotics can be synthesized or natural. The natural antibiotics are generally produced by two groups of fungi, *Penicillium* and *Cephalosporium* molds. The β-lactam antibiotics produced by *Penicillium* are the natural penicillins, such as penicillin G or penicillin V. These are produced by fermentation of *Penicillium chrysogenum*. The natural penicillins have a narrow spectrum of activity and are generally effective against *Streptococcus*, *Gonococcus*, and *Staphylococcus*. Other types of natural penicillins, which are also effective against gram-positive bacteria, include penicillins F, X, K, and O.

Semi-synthetic penicillins are generally modifications of the molecule 6-aminopenicillanic acid produced by a mold. The 6-aminopenicillanic acid can be modified by addition of side chains which produce penicillins having broader spectrums of activity than natural penicillins or various other advantageous properties. Some types of semi-synthetic penicillins have broad spectrums against gram-positive and gram-negative bacteria, but are inactivated by penicillinase. These semi-synthetic penicillins include ampicillin, carbenicillin, oxacillin, azlocillin, mezlocillin, and piperacillin. Other types of semi-synthetic penicillins have narrower activities against gram-positive bacteria, but have developed properties such that they are not inactivated by penicillinase. These include, for instance, methicillin, dicloxacillin, and nafcillin. Some of the broad spectrum semi-synthetic penicillins can be used in combination with β-lactamase inhibitors, such as clavulanic acids and sulbactam. The β-lactamase inhibitors do not have anti-microbial action but they function to inhibit penicillinase, thus protecting the semi-synthetic penicillin from degradation.

Another type of β-lactam antibiotic is the cephalosporins. Cephalosporins are produced by *Cephalosporium* molds, and have a similar mode of action to penicillin. They are sensitive to degradation by bacterial β-lactamases, and thus, are not always effective alone. Cephalolsporins, however, are resistant to penicillinase. They are effective against a variety of gram-positive and gram-negative bacteria. Cephalolsporins include, but are not

limited to, cephalothin, cephapirin, cephalexin, cefamandole, cefaclor, cefazolin, cefuroxine, cefoxitin, cefotaxime, cefsulodin, cefetamet, cefixime, ceftriaxone, cefoperazone, ceftazidine, and moxalactam.

Bacitracin is another class of antibiotics which inhibit cell wall synthesis. These antibiotics, produced by *Bacillus* species, prevent cell wall growth by inhibiting the release of muropeptide subunits or peptidoglycan from the molecule that delivers the subunit to the outside of the membrane. Although bacitracin is effective against gram-positive bacteria, its use is limited in general to topical administration because of its high toxicity. Since lower effective doses of bacitracin can be used when the compound is administered with the aziridino compounds in accordance with the invention, this compound can be used systemically and the toxicity reduced.

Carbapenems are another type of broad spectrum β-lactam antibiotic, which is capable of inhibiting cell wall synthesis. Examples of carbapenems include, but are not limited to, imipenems. Monobactems are also broad spectrum β-lactam antibiotics, and include, euztreonam. An antibiotic produced by *Streptomyces*, vancomycin, is also effective against gram-positive bacteria by inhibiting cell membrane synthesis.

Another class of anti-bacterial agents is the anti-bacterial agents that are cell membrane inhibitors. These compounds disorganize the structure or inhibit the function of bacterial membranes. Alteration of the cytoplasmic membrane of bacteria results in leakage of cellular materials from the cell. Compounds that inhibit or interfere with the cell membrane cause death of the cell because the integrity of the cytoplasmic and outer membranes is vital to bacteria. One problem with anti-bacterial agents that are cell membrane inhibitors is that they can produce effects in eukaryotic cells as well as bacteria because of the similarities in phospholipids in bacterial and eukaryotic membranes. Thus these compounds are rarely specific enough to permit these compounds to be used systemically and prevent the use of high doses for local administration.

One clinically useful anti-bacterial agent that is a cell membrane inhibitor is Polymyxin, produced by *Bacillus polymyxis*. Polymyxins interfere with membrane function by binding to membrane phospholipids. Polymyxin is effective mainly against Gram-negative bacteria and is generally used in severe *Pseudomonas* infections or *Pseudomonas* infections that are resistant to less toxic antibiotics. It is also used in some limited instances topically. The limited use of this agent is due to the severe side effects associated with systemic administration, such as damage to the kidney and other organs.

Other cell membrane inhibitors include Amphotericin B and Nystatin produced by the bacterium *Streptomyces* which are also anti-fungal agents, used predominantly in the treatment of systemic fungal infections and *Candida* yeast infections respectively. Imidazoles, produced by the bacterium *Streptomyces*, are another class of antibiotic that is a cell membrane inhibitor. Imidazoles are used as bacterial agents as well as anti-fungal agents, e.g., used for treatment of yeast infections, dermatophytic infections, and systemic fungal infections. Imidazoles include but are not limited to clotrimazole, miconazole, ketoconazole, itraconazole, and fluconazole.

Many anti-bacterial agents are protein synthesis inhibitors. These compounds prevent bacteria from synthesizing structural proteins and enzymes and thus cause inhibition of bacterial cell growth or function or cell death. In general these compounds interfere with the processes of transcription or translation. Anti-bacterial agents that block transcription include but are not limited to Rifampins, produced by the bacterium *Streptomyces* and Ethambutol, a synthetic chemical. Rifampins, which inhibit the enzyme RNA polymerase, have a broad spectrum activity and are effective against gram-positive and gram-negative bacteria as well as *Mycobacterium tuberculosis*. Ethambutol is effective against *Mycobacterium tuberculosis*.

Anti-bacterial agents which block translation interfere with bacterial ribosomes to prevent mRNA from being translated into proteins. In general this class of compounds includes but is not limited to tetracyclines, chloramphenicol, the macrolides (e.g. erythromycin) and the aminoglycosides (e.g. streptomycin).

Some of these compounds bind irreversibly to the 30S ribosomal subunit and cause a misreading of the mRNA, e.g., the aminoglycosides. The aminoglycosides are a class of antibiotics which are produced by the bacterium *Streptomyces,* such as, for instance streptomycin, kanamycin, tobramycin, amikacin, and gentamicin. Aminoglycosides have been used against a wide variety of bacterial infections caused by Gram-positive and Gram-negative bacteria. Streptomycin has been used extensively as a primary drug in the treatment of tuberculosis. Gentamicin is used against many strains of Gram-positive and Gram-negative bacteria, including *Pseudomonas* infections, especially in combination with tobramycin. Kanamycin is used against many Gram-positive bacteria, including penicillin-resistant *Staphylococci*. One side effect of aminoglycosides that has limited their use clinically is that at dosages which are essential for efficacy, prolonged use has been shown to impair kidney function and cause damage to the auditory nerves leading to deafness.

Another type of translation inhibitor anti-bacterial agent is the tetracyclines. The tetracyclines bind reversibly to the 30s ribosomal subunit and interfere with the binding of charged tRNA to the bacterial ribosome. The tetracyclines are a class of antibiotics, produced by the bacterium *Streptomyces,* that are broad-spectrum and are effective against a variety of gram-positive and gram-negative bacteria. Examples of tetracyclines include tetracycline, minocycline, doxycycline, and chlortetracycline. They are important for the treatment of many types of bacteria but are particularly important in the treatment of Lyme disease.

Anti-bacterial agents such as the macrolides bind reversibly to the 50S ribosomal subunit and inhibits elongation of the protein by peptidyl transferase or prevents the release of uncharged tRNA from the bacterial ribosome or both. The macrolides contain large lactone rings linked through glycoside bonds with amino sugars. These compounds include erythromycin, roxithromycin, clarithromycin, oleandomycin, and azithromycin. Erythromycin is active against most Gram-positive bacteria, *Neisseria*, *Legionella* and *Haemophilus*, but not against the *Enterobacteriaceae*. Lincomycin and clindamycin, which block peptide bond formation during protein synthesis, are used against gram-positive bacteria.

Another type of translation inhibitor is chloramphenicol. Chloramphenicol binds the 70S ribosome inhibiting the bacterial enzyme peptidyl transferase thereby preventing the growth of the polypeptide chain during protein synthesis. Chloramphenicol can be prepared from *Streptomyces* or produced entirely by chemical synthesis. One serious side effect associated with chloramphenicol is aplastic anemia. Aplastic anemia develops at doses of chloramphenicol which are effective for treating bacteria in a small proportion (1/50,000) of patients. Chloramphenicol which was once a highly prescribed antibiotic is now seldom uses as a result of the deaths from anemia. Because of its effectiveness it is still used in life-threatening situations (e.g. typhoid fever). By combining chloramphenicol with aziridino compounds as described herein, chloramphenicol can again be used as an anti-bacterial agent because the action of the aziridino compounds allows a lower dose of the chloramphenicol to be used, a dose that does not produce side effects.

Some anti-bacterial agents disrupt nucleic acid synthesis or function, e.g., bind to DNA or RNA so that their messages cannot be read. These include but are not limited to quinolones and co-trimoxazole, both synthetic chemicals and rifamycins, a natural or semi-synthetic chemical. The quinolones block bacterial DNA replication by inhibiting the DNA

gyrase, the enzyme needed by bacteria to produce their circular DNA. They are broad spectrum and examples include norfloxacin, ciprofloxacin, enoxacin, nalidixic acid and temafloxacin. Nalidixic acid is a bactericidal agent that binds to the DNA gyrase enzyme (topoisomerase) which is essential for DNA replication and allows supercoils to be relaxed and reformed, inhibiting DNA gyrase activity. The main use of nalidixic acid is in treatment of lower urinary tract infections (UTI) because it is effective against several types of Gram-negative bacteria such as *E. coli*, *Enterobacter aerogenes*, *K. pneumoniae* and *Proteus* species which are common causes of UTI. Co-trimoxazole is a combination of sulfamethoxazole and trimethoprim, which blocks the bacterial synthesis of folic acid needed to make DNA nucleotides. Rifampicin is a derivative of rifamycin that is active against Gram-positive bacteria (including *Mycobacterium tuberculosis* and meningitis caused by *Neisseria meningitidis*) and some Gram-negative bacteria. Rifampicin binds to the beta subunit of the polymerase and blocks the addition of the first nucleotide which is necessary to activate the polymerase, thereby blocking mRNA synthesis.

Another class of anti-bacterial agents is compounds that function as competitive inhibitors of bacterial enzymes. The competitive inhibitors are mostly all structurally similar to a bacterial growth factor and compete for binding but do not perform the metabolic function in the cell. These compounds include sulfonamides and chemically modified forms of sulfanilamide which have even higher and broader antibacterial activity. The sulfonamides (e.g. gantrisin and trimethoprim) are useful for the treatment of *Streptococcus pneumoniae*, beta-hemolytic *streptococci* and *E. coli*, and have been used in the treatment of uncomplicated UTI caused by E. coli, and in the treatment of meningococcal meningitis.

Antiviral agents are compounds which prevent infection of cells by viruses or replication of the virus within the cell. There are many fewer antiviral drugs than antibacterial drugs because the process of viral replication is so closely related to DNA replication within the host cell, that non-specific antiviral agents would often be toxic to the host. There are several stages within the process of viral infection which can be blocked or inhibited by antiviral agents. These stages include, attachment of the virus to the host cell (immunoglobulin or binding peptides), uncoating of the virus (e.g. amantadine), synthesis or translation of viral mRNA (e.g. interferon), replication of viral RNA or DNA (e.g. nucleoside analogues), maturation of new virus proteins (e.g. protease inhibitors), and budding and release of the virus.

Nucleotide analogues are synthetic compounds which are similar to nucleotides, but which have an incomplete or abnormal deoxyribose or ribose group. Once the nucleotide analogues are in the cell, they are phosphorylated, producing the triphosphate formed which competes with normal nucleotides for incorporation into the viral DNA or RNA. Once the triphosphate form of the nucleotide analogue is incorporated into the growing nucleic acid chain, it causes irreversible association with the viral polymerase and thus chain termination. Nucleotide analogues include, but are not limited to, acyclovir (used for the treatment of herpes simplex virus and varicella-zoster virus), gancyclovir (useful for the treatment of cytomegalovirus), idoxuridine, ribavirin (useful for the treatment of respiratory syncitial virus), dideoxyinosine, dideoxycytidine, and zidovudine (azidothymidine).

The interferons are cytokines which are secreted by virus-infected cells as well as immune cells. The interferons function by binding to specific receptors on cells adjacent to the infected cells, causing the change in the cell which protects it from infection by the virus. α and β-interferon also induce the expression of Class I and Class II MHC molecules on the surface of infected cells, resulting in increased antigen presentation for host immune cell recognition. α and β-interferons are available as recombinant forms and have been used for the treatment of chronic hepatitis B and C infection. At the dosages which are effective for anti-viral therapy, interferons have severe side effects such as fever, malaise and weight loss.

Immunoglobulin therapy is used for the prevention of viral infection. Immunoglobulin therapy for viral infections is different than bacterial infections, because rather than being antigen-specific, the immunoglobulin therapy functions by binding to extracellular virions and preventing them from attaching to and entering cells which are susceptible to the viral infection. The therapy is useful for the prevention of viral infection for the period of time that the antibodies are present in the host. In general there are two types of immunoglobulin therapies, normal immunoglobulin therapy and hyper-immunoglobulin therapy. Normal immune globulin therapy utilizes a antibody product which is prepared from the serum of normal blood donors and pooled. This pooled product contains low titers of antibody to a wide range of human viruses, such as hepatitis A, parvovirus, enterovirus (especially in neonates). Hyper-immune globulin therapy utilizes antibodies which are prepared from the serum of individuals who have high titers of an antibody to a particular virus. Those antibodies are then used against a specific virus. Examples of hyper-immune globulins include zoster immune globulin (useful for the prevention of varicella in immuno-compromised children and neonates), human rabies

immunoglobulin (useful in the post-exposure prophylaxis of a subject bitten by a rabid animal), hepatitis B immune globulin (useful in the prevention of hepatitis B virus, especially in a subject exposed to the virus), and RSV immune globulin (useful in the treatment of respiratory syncitial virus infections).

Another type of immunoglobulin therapy is active immunization. This involves the administration of antibodies or antibody fragments to viral surface proteins. Two types of vaccines which are available for active immunization of hepatitis B include serum-derived hepatitis B antibodies and recombinant hepatitis B antibodies. Both are prepared from HBsAg. The antibodies are administered in three doses to subjects at high risk of infection with hepatitis B virus, such as health care workers, sexual partners of chronic carriers, and infants.

Thus antiviral agents that can be combined with aziridino compounds in the therapeutic compositions of the invention include nucleoside analogs, nonnucleoside reverse transcriptase inhibitors, protease inhibitors, and integrase inhibitors. Specific examples of antiviral compounds include the following: Acemannan; Acyclovir; Acyclovir Sodium; Adefovir; Alovudine; Alvircept Sudotox; Amantadine Hydrochloride; Aranotin; Arildone; Atevirdine Mesylate; Avridine; Cidofovir; Cipamfylline; Cytarabine Hydrochloride; Delavirdine Mesylate; Desciclovir; Didanosine; Disoxaril; Edoxudine; Enviradene; Enviroxime; Famciclovir; Famotine Hydrochloride; Fiacitabine; Fialuridine; Fosarilate; Foscarnet Sodium; Fosfonet Sodium; Ganciclovir; Ganciclovir Sodium; Idoxuridine; Indinavir; Kethoxal; Lamivudine; Lobucavir; Memotine Hydrochloride; Methisazone; Nelfinavir; Nevirapine; Penciclovir; Pirodavir; Ribavirin; Rimantadine Hydrochloride; Ritonavir; Saquinavir Mesylate; Somantadine Hydrochloride; Sorivudine; Statolon; Stavudine; Tilorone Hydrochloride; Trifluridine; Valacyclovir Hydrochloride; Vidarabine; Vidarabine Phosphate; Vidarabine Sodium Phosphate; Viroxime; Zalcitabine; Zidovudine; Zinviroxime and integrase inhibitors.

Parasiticides are agents that kill parasites directly. Such compounds are known in the art and are generally commercially available. Examples of parasiticides useful for human administration include but are not limited to albendazole, amphotericin B, benznidazole, bithionol, chloroquine HCl, chloroquine phosphate, clindamycin, dehydroemetine, diethylcarbamazine, diloxanide furoate, eflornithine, furazolidaone, glucocorticoids, halofantrine, iodoquinol, ivermectin, mebendazole, mefloquine, meglumine antimoniate, melarsoprol, metrifonate, metronidazole, niclosamide, nifurtimox, oxamniquine,

paromomycin, pentamidine isethionate, piperazine, praziquantel, primaquine phosphate, proguanil, pyrantel pamoate, pyrimethanmine-sulfonamides, pyrimethanmine-sulfadoxine, quinacrine HCl, quinine sulfate, quinidine gluconate, spiramycin, stibogluconate sodium (sodium antimony gluconate), suramin, tetracycline, doxycycline, thiabendazole, tinidazole, trimethroprim-sulfamethoxazole, and tryparsamide some of which are used alone or in combination with others.

Parasiticides used in non-human subjects include piperazine, diethylcarbamazine, thiabendazole, fenbendazole, albendazole, oxfendazole, oxibendazole, febantel, levamisole, pyrantel tartrate, pyrantel pamoate, dichlorvos, ivermectin, doramectic, milbemycin oxime, iprinomectin, moxidectin, N-butyl chloride, toluene, hygromycin B thiacetarsemide sodium, melarsomine, praziquantel, epsiprantel, benzimidazoles such as fenbendazole, albendazole, oxfendazole, clorsulon, albendazole, amprolium; decoquinate, lasalocid, monensin sulfadimethoxine; sulfamethazine, sulfaquinoxaline, metronidazole.

Parasiticides used in horses include mebendazole, oxfendazole, febantel, pyrantel, dichlorvos, trichlorfon, ivermectin, piperazine; for *S. westeri*: ivermectin, benzimiddazoles such as thiabendazole, cambendazole, oxibendazole and fenbendazole. Useful parasiticides in dogs include milbemycin oxine, ivermectin, pyrantel pamoate and the combination of ivermectin and pyrantel. The treatment of parasites in swine can include the use of levamisole, piperazine, pyrantel, thiabendazole, dichlorvos and fenbendazole. In sheep and goats anthelmintic agents include levamisole or ivermectin. Caparsolate has shown some efficacy in the treatment of *D. immitis* (heartworm) in cats.

Agents used in the prevention and treatment of protozoal diseases in poultry, particularly trichomoniasis, can be administered in the feed or in the drinking water and include protozoacides such as aminonitrothiazole, dimetridazole (Emtryl), nithiazide (Hepzide) and Enheptin.

Anti-fungal agents are useful for the treatment and prevention of infective fungi. Anti-fungal agents are sometimes classified by their mechanism of action. Some anti-fungal agents function as cell wall inhibitors by inhibiting glucose synthase. These include, but are not limited to, basiungin/ECB. Other anti-fungal agents function by destabilizing membrane integrity. These include, but are not limited to, imidazoles, such as clotrimazole, sertaconzole, fluconazole, itraconazole, ketoconazole, miconazole, and voriconacole, as well as FK 463, amphotericin B, BAY 38-9502, MK 991, pradimicin, UK 292, butenafine, and

terbinafine. Other anti-fungal agents function by breaking down chitin (e.g. chitinase) or immunosuppression (501 cream).

Some exemplary anti-fungal agents include imidazoles, FK 463, amphotericin B, BAY 38-9502, MK 991, pradimicin, UK 292, butenafine, chitinase and 501 cream, Acrisorcin; Ambruticin; Amorolfine, Amphotericin B; Azaconazole; Azaserine; Basifungin; Bifonazole; Biphenamine Hydrochloride; Bispyrithione Magsulfex; Butoconazole Nitrate; Calcium Undecylenate; Candicidin; Carbol-Fuchsin; Chlordantoin; Ciclopirox; Ciclopirox Olamine; Cilofungin; Cisconazole; Clotrimazole; Cuprimyxin; Denofungin; Dipyrithione; Doconazole; Econazole; Econazole Nitrate; Enilconazole; Ethonam Nitrate; Fenticonazole Nitrate; Filipin; Fluconazole; Flucytosine; Fungimycin; Griseofulvin; Hamycin; Isoconazole; Itraconazole; Kalafungin; Ketoconazole; Lomofungin; Lydimycin; Mepartricin; Miconazole; Miconazole Nitrate; Monensin; Monensin Sodium; Naftifine Hydrochloride; Neomycin Undecylenate; Nifuratel; Nifurmerone; Nitralamine Hydrochloride; Nystatin; Octanoic Acid; Orconazole Nitrate; Oxiconazole Nitrate; Oxifungin Hydrochloride; Parconazole Hydrochloride; Partricin; Potassium Iodide; Proclonol; Pyrithione Zinc; Pyrrolnitrin; Rutamycin; Sanguinarium Chloride; Saperconazole; Scopafungin; Selenium Sulfide; Sinefungin; Sulconazole Nitrate; Terbinafine; Terconazole; Thiram; Ticlatone; Tioconazole; Tolciclate; Tolindate; Tolnaftate; Triacetin; Triafungin; Undecylenic Acid; Viridofulvin; Zinc Undecylenate; and Zinoconazole Hydrochloride.

Neoplastic diseases include various types of cancer, including but not limited to: biliary tract cancer; bladder cancer; breast cancer; brain cancer including glioblastomas and medulloblastomas; cervical cancer; choriocarcinoma; colon cancer; endometrial cancer; esophageal cancer; gastric cancer; head and neck cancer; hematological neoplasms including acute lymphocytic and myelogenous leukemia, multiple myeloma, AIDS-associated leukemias and adult T-cell leukemia lymphoma; intraepithelial neoplasms including Bowen's disease and Paget's disease; liver cancer; lung cancer including small cell lung cancer and non-small cell lung cancer; lymphomas including Hodgkin's disease and lymphocytic lymphomas; neuroblastomas; oral cancer including squamous cell carcinoma; ovarian cancer including those arising from epithelial cells, stromal cells, germ cells and mesenchymal cells; pancreatic cancer; prostate cancer; rectal cancer; sarcomas including leiomyosarcoma, rhabdomyosarcoma, liposarcoma, fibrosarcoma, synovial sarcoma and osteosarcoma; skin cancer including melanoma, Kaposi's sarcoma, basocellular cancer, and squamous cell cancer; testicular cancer including germinal tumors such as seminoma, non-seminoma

(teratomas, choriocarcinomas), stromal tumors, and germ cell tumors; thyroid cancer including thyroid adenocarcinoma and medullar carcinoma; transitional cancer; renal cancer including adenocarcinoma and Wilms tumor; and B cell malignacies including B cell non-Hodgkin's lymphoma (NHL), B cell acute lymphocytic leukemia (B-ALL), B cell precursor acute lymphocytic leukemia (pre-B-ALL), B cell chronic lymphocytic leukemia (B-CLL), hairy cell leukemia, precursor B-lymphoblastic leukemia/lymphoma, prolymphocytic leukemia, small lymphocytic lymphoma, lymphoplasmacytoid lymphoma, immunocytoma, mantle cell lymphoma, follicular follicle center lymphoma, marginal zone B-cell lymphomas, splenic marginal zone lymphoma, hairy cell lymphoma, plasmacytoma, plasma cell myeloma, large B-cell lymphomas, and Burkett's lymphoma.

Antineoplastic compounds that can be used in combination with the aziridino compounds disclosed herein include, but are not limited to, the following sub-classes of compounds. Determination of dosages of antineoplastic compounds to be administered in combination with aziridino compounds for particular cancers is well within routine experimentation for one of ordinary skill in the art.

Antineoplastic agents include: Acivicin; Aclarubicin; Acodazole Hydrochloride; Acronine; Adozelesin; Adriamycin; Aldesleukin ; Altretamine; Ambomycin; Ametantrone Acetate; Aminoglutethimide; Amsacrine; Anastrozole; Anthramycin; Asparaginase; Asperlin; Azacitidine; Azetepa; Azotomycin; Batimastat; Benzodepa; Bicalutamide; Bisantrene Hydrochloride; Bisnafide Dimesylate; Bizelesin; Bleomycin Sulfate; Brequinar Sodium; Bropirimine ; Busulfan; Cactinomycin; Calusterone; Caracemide; Carbetimer; Carboplatin; Carmustine; Carubicin Hydrochloride; Carzelesin; Cedefingol; Chlorambucil; Cirolemycin ; Cisplatin; Cladribine; Crisnatol Mesylate; Cyclophosphamide ; Cytarabine ; Dacarbazine; DACA (N-[2-(Dimethyl-amino)ethyl]acridine-4-carboxamide); Dactinomycin; Daunorubicin Hydrochloride; Daunomycin; Decitabine; Dexormaplatin; Dezaguanine; Dezaguanine Mesylate; Diaziquone; Docetaxel; Doxorubicin; Doxorubicin Hydrochloride; Droloxifene; Droloxifene Citrate; Dromostanolone Propionate; Duazomycin; Edatrexate; Eflornithine Hydrochloride ; Elsamitrucin; Enloplatin; Enpromate; Epipropidine; Epirubicin Hydrochloride; Erbulozole; Esorubicin Hydrochloride; Estramustine; Estramustine Phosphate Sodium; Etanidazole; Ethiodized Oil I 131; Etoposide; Etoposide Phosphate; Etoprine; Fadrozole Hydrochloride; Fazarabine; Fenretinide; Floxuridine; Fludarabine Phosphate; Fluorouracil; 5-FdUMP; Flurocitabine; Fosquidone; Fostriecin Sodium; Gemcitabine; Gemcitabine Hydrochloride; Gleevec; Gold Au 198 ; Hydroxyurea; Idarubicin

Hydrochloride; Ifosfamide; Ilmofosine; Interferon Alfa-2a ; Interferon Alfa-2b ; Interferon Alfa-n1; Interferon Alfa-n3; Interferon Beta- I a ; Interferon Gamma- I b; Iproplatin; Irinotecan Hydrochloride ; Lanreotide Acetate; Letrozole; Leuprolide Acetate ; Liarozole Hydrochloride; Lometrexol Sodium; Lomustine; Losoxantrone Hydrochloride; Masoprocol; Maytansine; Mechlorethamine Hydrochloride; Megestrol Acetate; Melengestrol Acetate; Melphalan; Menogaril; Mercaptopurine; Methotrexate; Methotrexate Sodium; Metoprine; Meturedepa; Mitindomide; Mitocarcin; Mitocromin; Mitogillin; Mitomalcin; Mitomycin; Mitosper; Mitotane; Mitoxantrone Hydrochloride; Mycophenolic Acid; Nocodazole; Nogalamycin; Ormaplatin; Oxisuran; Paclitaxel; Pegaspargase; Peliomycin; Pentamustine; Peplomycin Sulfate; Perfosfamide; Pipobroman; Piposulfan; Piroxantrone Hydrochloride; Plicamycin; Plomestane; Porfimer Sodium; Porfiromycin ; Prednimustine; Procarbazine Hydrochloride; Puromycin; Puromycin Hydrochloride; Pyrazofurin; Riboprine; Rogletimide; Safingol ; Safingol Hydrochloride ; Semustine; Simtrazene; Sparfosate Sodium; Sparsomycin; Spirogermanium Hydrochloride; Spiromustine; Spiroplatin; Streptonigrin; Streptozocin; Strontium Chloride Sr 89; Sulofenur; Talisomycin; Taxane; Taxoid; Tecogalan Sodium; Tegafur; Teloxantrone Hydrochloride; Temoporfin; Teniposide; Teroxirone; Testolactone; Thiamiprine; Thioguanine; Thiotepa; Thymitaq; Tiazofurin; Tirapazamine; Tomudex; TOP-53; Topotecan Hydrochloride; Toremifene Citrate; Trestolone Acetate; Triciribine Phosphate; Trimetrexate; Trimetrexate Glucuronate; Triptorelin; Tubulozole Hydrochloride; Uracil Mustard; Uredepa; Vapreotide; Verteporfin; Vinblastine; Vinblastine Sulfate; Vincristine; Vincristine Sulfate; Vindesine; Vindesine Sulfate; Vinepidine Sulfate; Vinglycinate Sulfate; Vinleurosine Sulfate; Vinorelbine Tartrate; Vinrosidine Sulfate; Vinzolidine Sulfate; Vorozole; Zeniplatin; Zinostatin; Zorubicin Hydrochloride; 2-Chlorodeoxyadenosine; 2'-Deoxyformycin; 9-aminocamptothecin; raltitrexed; N-propargyl-5,8-dideazafolic acid; 2-chloro-2'-arabino-fluoro-2'-deoxyadenosine; 2-chloro-2'-deoxyadenosine; anisomycin; trichostatin A; hPRL-G129R; CEP-751; linomide.

Other anti-neoplastic compounds include: 20-epi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid;

ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives (e.g., 10-hydroxy- camptothecin); canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetrorelix; chlorins; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrodidemnin B; deslorelin; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, 9-; dioxamycin; diphenyl spiromustine; discodermolide; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epothilones (A, R = H; B, R = Me); epithilones; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide; etoposide 4'-phosphate (etopofos); exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorunicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; irinotecan; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide + estrogen + progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol;

lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mithracin; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A + myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone + pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; podophyllotoxin; porfimer sodium; porfiromycin; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen binding protein; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor;

stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thalidomide; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene dichloride; topotecan; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; zinostatin stimalamer.

Anti-cancer Supplementary Potentiating Agents include: Tricyclic anti-depressant drugs (e.g., imipramine, desipramine, amitryptyline, clomipramine, trimipramine, doxepin, nortriptyline, protriptyline, amoxapine and maprotiline); non-tricyclic anti-depressant drugs (e.g., sertraline, trazodone and citalopram); Ca^{++} antagonists (e.g., verapamil, nifedipine, nitrendipine and caroverine); Calmodulin inhibitors (e.g., prenylamine, trifluoroperazine and clomipramine); Amphotericin B; Triparanol analogues (e.g., tamoxifen); antiarrhythmic drugs (e.g., quinidine); antihypertensive drugs (e.g., reserpine); Thiol depleters (e.g., buthionine and sulfoximine) and Multiple Drug Resistance reducing agents such as Cremaphor EL. The compounds of the invention also can be administered with cytokines such as granulocyte colony stimulating factor.

Antiproliferative agent: Piritrexim Isethionate.

Antiprostatic hypertrophy: Sitogluside.

Benign prostatic hyperplasia therapy agent: Tamsulosin Hydrochloride.

Prostate growth inhibitor: Pentomone.

Angiogenesis inhibitors: Endostatin, angiostatin, soluble troponin I.

Radioactive agents include: Fibrinogen I 125 ; Fludeoxyglucose F 18 ; Fluorodopa F 18 ; Insulin I 125; Insulin I 131; Iobenguane I 123; Iodipamide Sodium I 131 ; Iodoantipyrine I 131 ; Iodocholesterol I 131 ; Iodohippurate Sodium I 123 ; Iodohippurate Sodium I 125 ; Iodohippurate Sodium I 131 ; Iodopyracet I 125 ; Iodopyracet I 131 ; Iofetamine Hydrochloride I 123 ; Iomethin I 125 ; Iomethin I 131 ; Iothalamate Sodium I 125 ;

Iothalamate Sodium I 131 ; Iotyrosine I 131; Liothyronine I 125; Liothyronine I 131; Merisoprol Acetate Hg 197; Merisoprol Acetate Hg 203; Merisoprol Hg 197 ; Selenomethionine Se 75 ; Technetium Tc 99m Antimony Trisulfide Colloid; Technetium Tc 99m Bicisate ; Technetium Tc 99m Disofenin ; Technetium Tc 99m Etidronate ; Technetium Tc 99m Exametazime ; Technetium Tc 99m Furifosmin ; Technetium Tc 99m Gluceptate ; Technetium Tc 99m Lidofenin ; Technetium Tc 99m Mebrofenin ; Technetium Tc 99m Medronate ; Technetium Tc 99m Medronate Disodium; Technetium Tc 99m Mertiatide ; Technetium Tc 99m Oxidronate ; Technetium Tc 99m Pentetate; Technetium Tc 99m Pentetate Calcium Trisodium; Technetium Tc 99m Sestamibi ; Technetium Tc 99m Siboroxime ; Technetium Tc 99m Succimer ; Technetium Tc 99m Sulfur Colloid ; Technetium Tc 99m Teboroxime ; Technetium Tc 99m Tetrofosmin ; Technetium Tc 99m Tiatide; Thyroxine I 125; Thyroxine I 131; Tolpovidone I 131 ; Triolein I 125; Triolein I 131.

Disorders of immune system function include those mediated by lymphocytes including T cells and B cells. T cell-mediated diseases have been characterized by the induction of cytotoxic T-lymphocytes expressing the CD8 antigen on their cell surface and/or helper T cells expressing the CD4 antigen on their cell surface. These diseases include graft-versus-host disease, graft rejection, and autoimmune disorders. such as multiple sclerosis, rheumatoid arthritis, Graves disease, Addison's disease, polymyositis, insulin dependent diabetes, primary biliary cirrhosis, systemic lupus erythematosus, psoriasis, and scleroderma.

Graft-versus-host disease may occur when cells of the immune system such as stem cells or lymphocytes are transplanted into an allogeneic host, such as one genetically different at the major histocompatibility complex, which encodes cell surface antigens that give rise to strong immunological reactions. Transplants of cells of the immune system are made for treating certain forms of leukemia, aplastic anemia, and various immunodeficiency diseases. In order to prevent rejection of the foreign cells, the host is typically immunosuppressed, as with irradiation and/or immunosuppressive drugs. The transplanted immunocompetent cells recognize the host as foreign and mount an immune response directed against the host. In humans, the clinical manifestations of this graft-versus-host disease include fever, rash, anorexia, nausea, vomiting and watery or bloody diarrhea, weight loss and death.

It has also been reported that transfusion associated graft-versus-host disease can occur in immunocompetent transfusion recipients (Anderson, K. C., et al., New Eng. J. Med. 323: 315-321, 1990).

Autoimmune disorders may be caused by any cells that recognize a self cell or protein as foreign, thereby mounting an inappropriate autoimmune response. Autoimmune diseases include plasma cell disorders such as IgM polyneuropathies, immune thrombocytopenias, and autoimmune hemolytic anemias; Sjogren's syndrome; multiple sclerosis; rheumatoid arthritis; autoimmune lymphoproliferative syndrome (ALPS); sarcoidosis; systemic lupus erythematosus; bullous pemphigoid; myasthenia gravis; encephalomyelitis; Addison's disease; Graves' disease; scleroderma; polymyositis; insulin dependent diabetes mellitus; autoimmune uveoretinitis; inflammatory bowel diseases including ulcerative colitis; pemphigus vulgaris; autoimmune thyroiditis; primary biliary cirrhosis; psoriatic arthritis; exfoliative psoriatic dermatitis; postular psoriasis; autoimmune hemolytic anemia; mixed connective tissue disease; autoimmune thrombocytopenic purpura; and other cell mediated inflammatory, granulomatous, degenerative and atrophic disorders.

Standard experimental systems may be employed to optimize the method of the present invention, such as to determine effective amounts of the arizidino-containing compositions of the invention. For example, the effect of the arizidino compounds of the present invention on multiple sclerosis can be examined in the experimental allergic encephalomyelitis (EAE) model in rodents, which is a recognized and widely used animal model of multiple sclerosis. EAE is an antigen-induced T cell-mediated autoimmune disease directed against myelin basic protein (MBP). EAE is induced by parenteral administration of MBP and an adjuvant (such as Freund's complete adjuvant). This treatment induces either a monophasic or an exacerbating/remitting form of demyelinating disease (depending on the species of animal and details of administration) having the characteristics of multiple sclerosis. Such a model is recognized in the art to provide animal *in vivo* data which is known to correlate with human clinical efficacy.

In like manner, the arizidino compounds and compositions of the present invention can be tested by administration to animals afflicted experimental diabetes, using the well-known spontaneous diabetes models such as the BB rat strain or the NOD mouse strain. Alternatively, induced diabetes, for example streptozotocin-induced diabetes in mice, can be used as a model.

The effects of arizidino compounds and compositions of the present invention on rheumatoid arthritis may be examined using standard mouse models for collagen-induced arthritis, which are known to correlate with human clinical efficacy.

The aziridino compounds can be administered in combination with therapeutics used in the treatment of immune system disorders, such as immune system modulating compounds that are commonly employed to treat mammals afflicted with lymphocyte-mediated diseases. Due to the effectiveness of the aziridino compounds on these diseases, the dosages of the immune system modulating compounds may be lowered such that some or all of the undesired side effects are avoided. Alternative drug regimens can be employed when the immune system modulating compounds are administered in combination with the aziridino compounds of the present invention, for example, alternating doses of the compounds of the present invention and the immune system modulating compounds, thereby reducing the frequency of administration and toxic side effects of these agents. Non-limiting examples of the immune system modulating compounds that can be used in above combinations include immunosuppressive agents such as cyclosporin A, Imuran (azathioprine), Cytoxan, (cyclophosphamide), prednisone, methylprednisolone, OKT-3 monoclonal antibodies, and FK-506.

The term "effective amount" of an aziridino compound (optionally combined with other non-aziridino compounds as described herein) refers to the amount necessary or sufficient to realize a desired biologic effect. For example, an effective amount of an aziridino compound for treating or preventing infectious disease is that amount necessary to prevent the infection with the microorganism if the subject is not yet infected or is that amount necessary to prevent an increase in infected cells or microorganisms present in the subject or that amount necessary to decrease the amount of the infection that would otherwise occur in the absence of the aziridino compound. Combined with the teachings provided herein, by choosing among the various active compounds and weighing factors such as potency, relative bioavailability, patient body weight, severity of adverse side-effects and preferred mode of administration, an effective prophylactic or therapeutic treatment regimen can be planned which does not cause substantial toxicity and yet is entirely effective to treat the particular subject. The effective amount for any particular application can vary depending on such factors as the disease or condition being treated, the particular aziridino compound and/or additional non-aziridino agent being administered, the size of the subject, and/or the severity of the disease or condition.

In some embodiments of the invention, an aziridino compound and one or more non-aziridino compounds are administered in a synergistic amount effective to treat or prevent infectious disease, neoplastic disease or immune system disorders. A synergistic amount is

that amount which produces a physiological response that is greater than the sum of the individual effects of either agent alone. For instance, in some embodiments of the invention, the physiological effect is a reduction in the number of cells infected with a microorganism. A synergistic amount is that amount which produces a reduction in infected cells that is greater than the sum of the infected cells reduced by either the aziridino compound or the non-aziridino compound alone. In other embodiments, the physiological result is a reduction in the number of microorganisms in the body. The synergistic amount in this case is that amount which produces the reduction that is greater than the sum of the reduction produced by either the aziridino compound or the non-aziridino compound alone. In other embodiments the physiological result is a decrease in physiological parameters associated with the infection, e.g., fungal lesions or other symptoms. For instance, a diagnosis of urinary tract infection is based on the presence and quantification of bacteria in the urine when greater than 10^5 colonies per milliliter of microorganisms are detected in a mid-stream, clean-voided urine specimen. A reduction in this number to 10^3 and preferably to fewer than 10^2 bacterial colonies per milliliter indicates that the infection has been eradicated.

Subject doses of the compounds of the invention for parenteral administration, stated in terms of subject body weight, range typically from about 0.01 mg to 100 mg/kg/day, more typically from about 1 to 50 mg/kg/day. Subject dosages for non-systemic administration, e.g., for topical administration, such as application to skin and/or mucosal body surfaces are typically administered as a solution, lotion or cream at a concentration of about 0.001% to about 50% vol/vol, and more typically at a concentration of about 0.01% to about 10% vol./vol; or for administration through inhalation to the the respiratory tract, are typically administered as an aerosol with a concentration of about 0.01 to about 1000 parts per million (ppm).

The dosages of the aziridino compounds, when administered systemically, result in peak blood concentrations of at least about 0.1 μg/ml, more preferably at least about 0.25 μg/ml, more preferably at least about 0.5 μg/ml, more preferably at least about 0.75 μg/ml, more preferably at least about 1 μg/ml, more preferably at least about 2 μg/ml, more preferably at least about 3 μg/ml, more preferably at least about 4 μg/ml, more preferably at least about 5 μg/ml, more preferably at least about 7 μg/ml, and still more preferably at least about 10 μg/ml. Still higher peak blood concentrations of the aziridino compounds can be achieved through systemic adminstration, including at least about 20 μg/ml, at least about 30

μg/ml, at least about 40 μg/ml, at least about 50 μg/ml, at least about 100 μg/ml, at least about 200 μg/ml, at least about 300 μg/ml, at least about 400 μg/ml, and at least about 500 μg/ml.

The aziridino compounds can be administered also on the basis of volume/volume dosage. Preferred volume/volume concentrations include from about 0.0001% to about 0.03% vol./vol. (i.e., 0.0001%, 0.0002%, 0.0003%, 0.0004%, 0.0005%, 0.0006%, 0.0007%, 0.0008%, 0.0009%, 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008%, 0.009%, 0.010%, 0.011%, 0.012%, 0.013%, 0.014%, 0.015%, 0.016%, 0.017%, 0.018%, 0.019%, 0.02%, 0.021%, 0.022%, 0.023%, 0.024%, 0.025%, 0.026%,0.027%, 0.028%, 0.029%, 0.03%, and fractional amounts therebetween).

Higher blood concentrations, if necessary for effective treatment, may be achieved by administering greater dosages of the aziridino compounds, or administering doses of the compounds more frequently.

Therapeutic doses of the non-aziridino compounds for use in combination with the aziridino compounds are well known in the field of medicine for the treatment of disease. These dosages have been extensively described in references such as Remington's Pharmaceutical Sciences, 18th ed., 1990; as well as many other medical references relied upon by the medical profession as guidance for the treatment of disease.

In certain embodiments of the invention, the aziridino compounds are administered on a routine schedule. The non-aziridino compounds, if used in combination with the aziridino compounds, may also be administered on a routine schedule, but alternatively, may be administered as symptoms arise. A "routine schedule" as used herein, refers to a predetermined designated period of time. The routine schedule may encompass periods of time which are identical or which differ in length, as long as the schedule is predetermined. For instance, the routine schedule may involve administration of the aziridino compounds on once or more times per day, every two days, every three days, every four days, every five days, every six days, a weekly basis, a monthly basis or any set number of days or weeks there-between, every two months, three months, four months, five months, six months, seven months, eight months, nine months, ten months, eleven months, twelve months, etc. Any particular combination would be covered by the routine schedule as long as it is determined ahead of time that the appropriate schedule involves administration on a certain day.

In other aspects, the invention relates to kits that are useful in the treatment of disease. One kit of the invention includes a container housing one or more aziridino compounds and optionally contains a container housing one or more non-aziridino compounds and

instructions for timing of administration of these agent(s). Other kits include containers housing a mixture of aziridino compounds and non-aziridino compounds. Preferably, the aziridino compounds are provided for systemic administration, and the instructions accordingly provide for this. In an important embodiment, the container housing the aziridino compound(s) is a sustained release vehicle is used herein in accordance with its prior art meaning of any device which slowly releases the aziridino compound(s).

The pharmaceutical compositions containing one or more aziridino compounds are housed in at least one container. The container may be a single container housing all of the anti-microbial agent together or it may be multiple containers or chambers housing individual dosages of the anti-microbial agent, such as a blister pack. The kit also has instructions for timing of administration of the anti-microbial agent. The instructions would direct the subject having an infectious disease or at risk of an infectious disease to take the anti-microbial agent at the appropriate time. For instance, the appropriate time for delivery of the medicament may be as the symptoms occur. Alternatively, the appropriate time for administration of the medicament may be on a routine schedule such as monthly or yearly.

For any compound described herein a therapeutically effective amount can be initially determined from *in vitro* assays and/or based on known effective amounts for known agents. For instance, the effective amount of aziridino compounds useful for inhibiting microbial growth, or for inhibiting tumor cell growth, or for inhibiting lymphocyte activation or growth, can be assessed using standard *in vitro* assays. These assays can be used to determine an effective amount of the particular aziridino compound for a particular disease.

Therapeutically effective amounts can also be determined from animal models as will be well known to and routinely performed by one of ordinary skill in the art. The applied dose can be adjusted based on the relative bioavailability and potency of the administered compound. Adjusting the dose to achieve maximal efficacy based on the methods described above and other methods as are well-known in the art is well within the capabilities of the ordinarily skilled artisan. Doses of non-aziridino compounds can be adjusted when they are combined with aziridino compounds by routine experimentation, based on the teachings within the specification.

The formulations of the invention are administered as pharmaceutical compositions that contain pharmaceutically acceptable concentrations of salt, buffering agents, preservatives, compatible carriers, adjuvants, and optionally other therapeutic ingredients.

Aziridino compounds can be administered by any ordinary route for administering medications. "Administering" the pharmaceutical composition of the present invention may be accomplished by any means known to the skilled artisan. Preferred routes of administration include but are not limited to oral, parenteral, intramuscular, intranasal, topical, intratracheal, inhalation, ocular, vaginal, and rectal.

For oral administration, the compounds (i.e., aziridino compounds, optionally in combination with one or more non-aziridino compound therapeutic agents) can be formulated readily by combining the active compound(s) with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject to be treated. Pharmaceutical preparations for oral use can be obtained as solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Optionally the oral formulations may also be formulated in saline or buffers for neutralizing internal acid conditions or may be administered without any carriers.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene

glycols. In addition, stabilizers may be added. Microspheres formulated for oral administration may also be used. Such microspheres have been well defined in the art. All formulations for oral administration should be in dosages suitable for such administration. Oral formulations optionally can be enterically coated in accordance with conventional coatings known in the art.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention may be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of *e.g.* gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch. Techniques for preparing aerosol delivery systems are well known to those of skill in the art. Generally, such systems should utilize components which will not significantly impair the biological properties of the therapeutic (see, for example, Sciarra and Cutie, "Aerosols," in Remington's Pharmaceutical Sciences, 18th edition, 1990, pp 1694-1712; incorporated by reference). Those of skill in the art can readily determine the various parameters and conditions for producing aerosols without resort to undue experimentation.

The compounds, when it is desirable to deliver them systemically, may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion in amounts and for a time sufficient to deliver an effective amount of the compounds and compositions of the invention (i.e., to achieve an effective dose). Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Parenteral administration includes interarterial, oral (injection), subcutaneous, interperitoneal, intrathecal, intravesicular or intravenous administration.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active

compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active compounds may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal or vaginal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

Suitable liquid or solid pharmaceutical preparation forms are, for example, aqueous or saline solutions for inhalation, microencapsulated, encochleated, coated onto microscopic gold particles, contained in liposomes, nebulized, aerosols, pellets for implantation into the skin, or dried onto a sharp object to be scratched into the skin. The pharmaceutical compositions also include granules, powders, tablets, coated tablets, (micro)capsules, suppositories, syrups, emulsions, suspensions, creams, drops or preparations with protracted release of active compounds, in whose preparation excipients and additives and/or auxiliaries such as disintegrants, binders, coating agents, swelling agents, lubricants, flavorings, sweeteners or solubilizers are customarily used as described above. The pharmaceutical compositions are suitable for use in a variety of drug delivery systems. For a brief review of methods for drug delivery, see Langer, *Science* 249:1527-1533, 1990, which is incorporated herein by reference.

The aziridino compounds may be administered using time release, delayed release, or sustained release systems. Such systems can avoid repeated administrations of the compounds, increasing convenience to the subject and the physician. Many types of release delivery systems are available and known to those of ordinary skill in the art. They include polymer base systems such as poly(lactide-glycolide), copolyoxalates, polycaprolactones, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polyanhydrides. Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Patent 5,075,109. Delivery systems also include non-polymer systems that are: lipids including sterols such as cholesterol, cholesterol esters and fatty acids or neutral fats such as mono-di- and tri-glycerides; hydrogel release systems; silastic systems; peptide based systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants; and the like. Specific examples include, but are not limited to: (a) erosional systems in which an agent of the invention is contained in a form within a matrix such as those described in U.S. Patent Nos. 4,452,775, 4,675,189, and 5,736,152, and (b) diffusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Patent Nos. 3,854,480, 5,133,974 and 5,407,686. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

The aziridino compounds may be administered *per se* (neat) or in the form of a pharmaceutically acceptable salt. When used in medicine the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically acceptable salts thereof. Such salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulphuric, nitric, phosphoric, maleic, acetic, salicylic, p-toluene sulphonic, tartaric, citric, methane sulphonic, formic, malonic, succinic, naphthalene-2-sulphonic, and benzene sulphonic. Also, such salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts of the carboxylic acid group.

Suitable buffering agents include: acetic acid and a salt (1-2% w/v); citric acid and a salt (1-3% w/v); boric acid and a salt (0.5-2.5% w/v); and phosphoric acid and a salt (0.8-2% w/v). Suitable preservatives include benzalkonium chloride (0.003-0.03% w/v); chlorobutanol (0.3-0.9% w/v); parabens (0.01-0.25% w/v) and thimerosal (0.004-0.02% w/v).

The pharmaceutical compositions of the invention contain an effective amount of an aziridino compounds and optionally other therapeutic agents, optionally included in a

pharmaceutically-acceptable carrier. The term "pharmaceutically-acceptable carrier" means one or more compatible solid or liquid filler, dilutants or encapsulating substances which are suitable for administration to a human or other vertebrate animal. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being commingled with the compounds of the present invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficiency.

Examples

Example 1: Effect of Ethyleneimine Oligomer Treatment on the Survival of Different Cell Lines

The study is performed to compare the effect of ethyleneimine oligomer treatment on eukaryotic cell lines of different origin. Two cell lines are used in the study, Ramos and FRhK. Ramos is EBV-negative B-cell lymphoma derived from 3-year-old male Caucasian with histologic diagnosis of American Burkitt's lymphoma (Klein, 1974; Klein, 1975). Ramos cells are capable of forming colonies in semi-solid medium *in vitro* and tumors in the mice (Nilsson, 1977). FRhK is a continuous cell line obtained from kidney tissue of a normal rhesus (*Macaca mulatta*) female fetus (Wallack, 1973). FRhK cells do not grow as colonies in agarose gel and are unable to form tumors in mice.

FRhK cells are grown to early confluency in 6-well plates in Dulbecco's MEM medium supplemented with 10% FBS. At this stage each well contains approximately $2x10^5$ cells. Ramos cells are grown to early confluency in RPMI 1640 medium supplemented with 10% FBS, washed with HBSS, and resuspended in fresh RPMI 1640 supplemented with 5% FBS to final concentration ~10^5 cells/mL. The cell suspension is aliquoted and an ethyleneimine oligomer is added to achieve final concentrations of 0.025%, 0.0083%, 0.0028%, 0.0009%, and 0.0003% (V/V). For control purposes, Ramos cells are sham treated with 0.25M sodium phosphate. After gentle mixing by inverting, Ramos cells containing different concentrations of the ethyleneimine oligomer, as well as Sham Control, are added to monolayers of FRhK cells preliminary washed with HBSS. Mixed cultures are incubated at 37°C in a 5% CO_2 100% humidified incubator for 3 and 24 hours. After the incubation,

Ramos cells are separated from FRhK cells, washed 3 times with HBSS, resuspended in RPMI 1640 medium supplemented with 10% FBS, and plated into 96-well plates with a seeding concentration of approximately 10^3 cells/well. FRhK cells are trypsinized, washed three times with HBSS, resuspended in Dulbecco's MEM medium supplemented with 10% FBS, and plated into 96-well plates with a seeding concentration of approximately 10^3 cells/well. Both cell lines are maintained at 37°C in a 5% CO_2 100% humidified incubator. Cell morphology, viability and proliferation are visually monitored on daily basis. 24 hours after the treatment, no differences between control and treated Ramos cells are detected in samples treated with lowest (0.0003%) concentration of ethyleneimine oligomer, both for 3 and 24 hours of incubation. All other concentrations are cytocidal or cytotoxic. For FRhK cells, no significant differences between control and treated cells are detected in samples treated with 0.0003%, 0.0009%, 0.0025%, and 0.0083% ethyleneimine oligomer, both for 3 and 24 hours of treatment. Only samples treated with 0.025% ethyleneimine oligomer show strong cytotoxicity. Ten days after the treatment, no viable Ramos cells are detected in any treated samples, while a complete monolayer of FRhK cells is observed in samples treated with 0.0083% ethyleneimine oligomer or less.

Materials/Equipment

The B-lymphoid cell line Ramos RA 1 (ATCC # CRL-1596) and the epithelial-like continuous cell line FRhK (ATCC # CRL-1688) are obtained from ATCC. Culture media including fetal bovine serum (FBS; Gibco #10082-147); Dulbecco's minimal Eagle's medium (MEM; Gibco #11965-092); Hanks' balanced salt solution (HBSS; Gibco #14175-095); and RPMI 1640 (Gibco #11875-093) were obtained from Gibco/Life Technologies. Culture flasks (Corning #430641), culture plates (Costar #3598 and #3516), CO_2 incubator (Forma Scientific, model 3110), biosafety hood (Forma Scientific, type A/B3 model 1184), and phase-contrast microscope (Zeiss, Invertoscop, 451201) are obtained from the indicated vendors.

Procedure

Both cell lines are maintained in plastic 75-cm^2 flasks at 37°C in a 5% CO_2, 100% humidity incubator. Growth medium consists of Dulbecco's MEM (FRhK) or RPMI 1640 (Ramos), both supplemented with 10% FBS. Before the experiment, freshly confluent FRhK cells are detached using trypsin and plated into 6-well plates with a split ratio of 1:2. Ramos

cells are transferred into 75-cm^2 flasks with a split ratio of 1:4. When FRhK cells reach early confluency (approximately $2x10^5$ cells/well), Ramos cells are collected from 75-cm^2 flasks and washed 3 times with HBSS. Each time cells are pelleted at 1,000 rpm for 10 min. After washing, Ramos cells are resuspended in RPMI 1640/5% FBS to final concentration of approximately 10^5 cells/mL. The cell suspension is aliquoted (2 mL each aliquot) and an ethyleneimine oligomer in 0.25M sodium phosphate is added to achieve final concentration of 0.025%, 0.0083%, 0.0028%, 0.0009%, and 0.0003% (V/V). Each concentration consists of three replicates. Sham Control samples receive 0.25M sodium phosphate only. After brief mixing, Ramos cells are added to FRhK cells in 6-well plates (2 mL/well), which are also washed with HBSS prior to addition of Ramos cells. Plates are incubated at 37°C in a 5% CO_2, 100% humidity incubator for 3 or 24 hours. After the incubation, Ramos cells are suspended by gentle pipeting, separated from FRhK cells, washed 3 times with HBSS, resuspended in fresh growth medium, and plated into 96-well plates with a seeding concentration of approximately 1,000 cells/well. FRhK cells are trypsinized, washed with HBSS, resuspended in growth medium, and also plated into 96-well plates with a seeding concentration of approximately 1,000 cells/well. 25% of the medium (0.5 mL) is changed daily. Cellular viability and proliferation (if any) is monitored under phase contrast on a daily basis using Zeiss Invertoskop microscope.

Results

Treatment of the cells with ehyleneimine oligomer causes significant cell death in case of Ramos cells while displaying only a mild effect on FRhK cells.

Based on these results, we conclude that low concentrations of an ethyleneimine oligomer effectively kills tumor cells. At the same conditions, the viability of normal cells and their ability to proliferate remain normal.

References

1. Klein G, Lindahl T, Jondal M, Leibold W, Menezes J, Nilsson K, Sundstrom C: Continuous lymphoid cell lines with characteristics of B cells (bone-marrow-derived), lacking the Eptstein-Barr virus genome and derived from three human lymphomas. Proc. Natl. Acad. Sci. USA, 71(8):3283-6. 1974.
2. Klein G, Giovanella B, Westman A, Stehlin JS, Munford D: An EBV-genome-negative cell line established from an American Burkitt lymphoma; receptor

characteristics, EBV infectibility and permanent conversion into EBV-positive sublines by *in vitro* infection. Intervirology, 5(6):319-34. 1975.

3. Dalton WS, Bergsagel PL, Kuehl WM, Anderson KC, Harousseau JL: Multiple Myeloma. Hematolog:157-77. 2001.

4. Appelbaum FR, Rowe JM, Radich J, Dick JE: Acute Myeloid Leukemia. Hematology:62-86. 2001.

5. Nillson K, Giovanella BC, Stehlin JS, Klein G: Tumorigenicity of human hematopoietic cell lines in athymic nude mice. Int. J. Cancer, 19(3):337-44. 1977.

6. Wallace RE, Vasington PJ, Petricciani JC, Hopps HE, Lorenz DE, Kadanka Z: Development and characterization of cell lines from subhuman primates. In Vitro, 8(5):333-41. 1973.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by examples provided, since the examples are intended as a single illustration of one aspect of the invention and other functionally equivalent embodiments are within the scope of the invention. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims. The advantages and objects of the invention are not necessarily encompassed by each embodiment of the invention.

All references, patents and patent publications that are recited in this application are incorporated in their entirety herein by reference.

We claim:

Claims

1. A method for treatment of a disease, comprising

preventing or inhibiting transcription and/or replication of a nucleic acid molecule by administering to a subject in need of such treatment an effective amount of one or more aziridino compounds.

2. The method of claim 1, wherein the nucleic acid molecule is a non-self nucleic acid molecule.

3. The method of claim 1, wherein the nucleic acid molecule is a mutated self nucleic acid molecule.

4. The method of claim 1, wherein the nucleic acid molecule is a leukocytic nucleic acid molecule.

5. The method of claim 1, wherein the nucleic acid molecule is RNA or DNA.

6. The method of claim 1, wherein the nucleic acid molecule is single-stranded or double-stranded.

7. A method for treating infectious disease, comprising

administering to a subject in need of such treatment an amount of an aziridino compound effective to treat the infectious disease by inhibiting the growth or replication of an infectious agent that causes the infectious disease.

8. A method for treating neoplastic disease, comprising

administering to a subject in need of such treatment an amount of an aziridino compound effective to treat the neoplastic disease by inhibiting the growth or replication of a cell or tumor that causes the neoplastic disease,

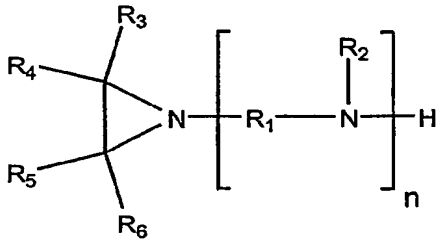
wherein the aziridino compound is not an aziridinylquinone compound.

9. A method for treating a disorder of immune system function, comprising

administering to a subject in need of such treatment an amount of an aziridino compound effective to treat the disorder of immune system function by inhibiting the growth or replication of an immune system cell.

10. The method of any of claims 7, 8 or 9, wherein the aziridino compound contains a linear alkyl group.

11 The method of claim 10, wherein the aziridino compound has the structure of formula II:

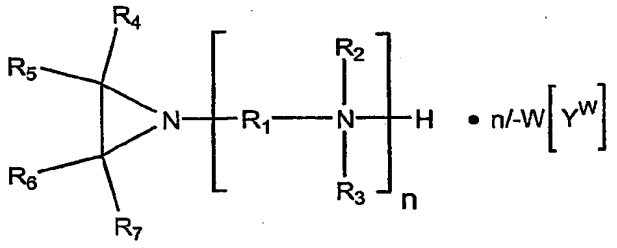


(II)

wherein each R_1 is a divalent hydrocarbon moiety containing between two and four carbon atoms, inclusive; each of R_2, R_3, R_4, R_5, and R_6 is, independently, H or a monovalent hydrocarbon moiety containing between one and four carbon atoms, inclusive; and n is an integer between one and ten, inclusive.

12. The method of claim 11, wherein R_2, R_3, R_4, R_5, and R_6 are H.

13. The method of claim 10, wherein the aziridino compound has the structure of formula III:



(III)

wherein each R_1 is a divalent hydrocarbon moiety containing between two and four carbon atoms, inclusive; each of R_2, R_3, R_4, R_5, R_6, and R_7 is, independently, H or a monovalent hydrocarbon moiety containing between one and four carbon atoms, inclusive; Y is pharmaceutically acceptable counter anion; W is the valency of Y; and n is an integer between one and ten, inclusive.

14. The method of claim 13, wherein R_2, R_3, R_4, R_5, and R_6 are H.

15. The method of claim 10, wherein the aziridino compound is an ethyleneimine oligomer.

16. The method of claim 15, wherein the ethyleneimine oligomer is an ethyleneimine dimer.

17. The method of claim 15, wherein the ethyleneimine oligomer is an ethyleneimine trimer.

18. The method of claim 7, further comprising administering to the subject an amount of one or more non-aziridino antimicrobial compounds effective to treat the infectious disease.

19. The method of claim 7, wherein the infectious disease is selected from the group consisting of viral infections, bacterial infections, parasite infections and fungal infections.

20. The method of claim 8, further comprising administering to the subject an amount of one or more non-aziridino antineoplastic compounds effective to treat the neoplastic disease.

21. The method of claim 9, further comprising administering to the subject an amount of one or more non-aziridino immune system modulating compounds effective to treat the disorder of immune system function.

22. The method of claim 9, wherein the subject has or is suspected of having a leukocyte mediated disease selected from the group consisting of autoimmune diseases, graft versus host diseases, allergy, T cell mediated diseases, and B cell mediated diseases.

23. The method of claim 22, wherein the disorder of immune system function is an autoimmune disease selected from the group consisting of multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, polymyositis, insulin dependent diabetes, primary biliary cirrhosis, systemic lupus erythematosus, psoriasis, autoimmune hemolytic anemia, mixed connective tissue disease, autoimmune thrombocytopenic purpura and scleroderma.

24. The method of any of claims 7, 8 or 9, wherein the aziridino compound is administered orally to the subject.

25. The method of claim 24, wherein the aziridino compound is formulated with an enteric coating.

26. The method of any of claims 7, 8 or 9, wherein the aziridino compound is administered parenterally to the subject.

27. The method of claim 26, wherein the parenteral administration is interarterial, oral, subcutaneous, interperitoneal, intrathecal, intravesicular or intravenous injection.

28. The method of any of claims 7, 8 or 9, wherein the aziridino compound is administered to the subject by implantation of a sustained release formulation.

29. The method of any of claims 7, 8 or 9, wherein the aziridino compound is administered to the subject in an amount effective to achieve a peak blood concentrations of at least about 0.1 μg/ml.

30. The method of claim 29, wherein the peak blood concentration of the aziridino compound is from about 1 μg/ml to about 500 μg/ml.

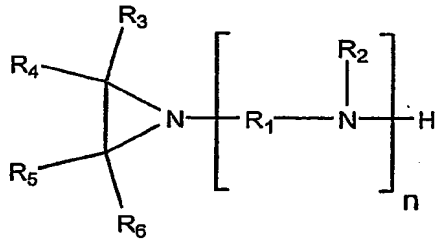
31. A pharmaceutical composition comprising,

a substantially pure preparation of an aziridino compound, wherein the aziridino compound is not an aziridinylquinone compound, and

a pharmaceutically acceptable carrier.

32. The pharmaceutical composition of claim 31, wherein the aziridino compound contains a linear alkyl group.

33. The pharmaceutical composition of claim 32, wherein the aziridino compound has the structure of formula II:

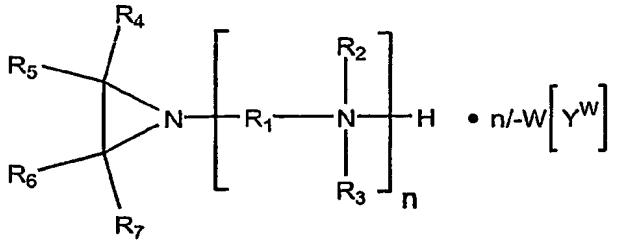


(II)

wherein each R_1 is a divalent hydrocarbon moiety containing between two and four carbon atoms, inclusive; each of R_2, R_3, R_4, R_5, and R_6 is, independently, H or a monovalent hydrocarbon moiety containing between one and four carbon atoms, inclusive; and n is an integer between one and ten, inclusive.

34. The pharmaceutical composition of claim 33, wherein R_2, R_3, R_4, R_5, and R_6 are H.

35. The pharmaceutical composition of claim 32, wherein the aziridino compound has the structure of formula III:



(III)

wherein each R_1 is a divalent hydrocarbon moiety containing between two and four carbon atoms, inclusive; each of R_2, R_3, R_4, R_5, R_6, and R_7 is, independently, H or a monovalent hydrocarbon moiety containing between one and four carbon atoms, inclusive; Y is pharmaceutically acceptable counter anion; W is the valency of Y; and n is an integer between one and ten, inclusive.

36. The pharmaceutical composition of claim 35, wherein R_2, R_3, R_4, R_5, and R_6 are H.

37. The pharmaceutical composition of claim 32, wherein the aziridino compound is an ethyleneimine oligomer.

38. The pharmaceutical composition of claim 37, wherein the ethyleneimine oligomer is an ethyleneimine dimer.

39. The pharmaceutical composition of claim 37, wherein the ethyleneimine oligomer is an ethyleneimine trimer.

40. The pharmaceutical composition of claim 31, wherein the composition is formulated for oral delivery.

41. The pharmaceutical composition of claim 31, wherein the composition is formulated for parenteral delivery.

42. The pharmaceutical composition of claim 31, further comprising one or more non-aziridino antimicrobial compounds.

43. The pharmaceutical composition of claim 31, further comprising one or more non-aziridino antineoplastic compounds.

44. The pharmaceutical composition of claim 31, further comprising one or more non-aziridino immune system modulating compounds.

45. A method for manufacturing a pharmaceutical composition, comprising

placing a substantially pure preparation of an aziridino compound, wherein the aziridino compound is not an aziridinylquinone compound, in a pharmaceutically acceptable carrier.

46. The method of claim 45, wherein the pharmaceutically acceptable carrier is suitable for oral administration.

47. The method of claim 46, further comprising the step of formulating the composition into a tablet or capsule.

48. The method of claim 47, wherein the tablet or capsule includes an enteric coating.

49. The method of claim 45, wherein the pharmaceutically acceptable carrier is suitable for parenteral administration.

50. The method of claim 49, further comprising the step of lyophilizing the composition to form a lyophilized preparation.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 August 2003 (14.08.2003)

PCT

(10) International Publication Number
WO 03/066806 A3

(51) International Patent Classification[7]: C12N 7/06, 7/00, A61K 39/00

(21) International Application Number: PCT/US02/35501

(22) International Filing Date:
5 November 2002 (05.11.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PCT/US01/49956
6 November 2001 (06.11.2001) US
60/365,648 18 March 2002 (18.03.2002) US
60/379,188 9 May 2002 (09.05.2002) US

(71) Applicant *(for all designated States except US)*: **V.I. TECHNOLOGIES, INC.** [US/US]; 134 Coolidge Avenue, Watertown, MA 02472 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **CHAPMAN, John** [US/US]; 77 Beaconwood Road #22, Newton, MA 02461 (US). **ALFORD, Bernadette** [US/US]; 11 Carter Drive, Framingham, MA 01701 (US). **PURMAL, Andrei, A.** [US/US]; 501 Lexington Street, Apt. 9, Waltham, MA 02452 (US). **ZAVIZION, Boris** [UA/US]; 44 Broadlawn Park #20, Chestnut Hill, MA 02467 (US).

(74) Agent: **VAN AMSTERDAM, John, R.**; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
24 December 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/066806 A3

(54) Title: THERAPEUTIC USE OF AZIRIDINO COMPOUNDS

(57) Abstract: Methods are provided for treatment of diseases. The methods include preventing or inhibiting transcription and/or replication of a nucleic acid molecule by administering to a subject in need of such treatment an effective amount of one or more aziridino compounds. Pharmaceutical compositions comprising the one or more aziridino compounds also are provided.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/35501

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12N 7/06; 7/00; A61K39/00
US CL : 435/238, 235.1, 226; 424/184.1; 514/642
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/238, 235.1, 226; 424/184.1; 514/642

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- A	US 6,093,564 A (BUDOWSKY et al) 25 July 2000(25.07.2000), see abstract and full text.	31-44 --- 1-30, 45-50
Y --- A	US 6,136,586 A (BUDOWSKI) 24 October 2000(24.10.2000), entire document.	31-44 --- 1-30, 45-50

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
25 August 2003 (25.08.2003)

Date of mailing of the international search report
12 SEP 2003

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
Facsimile No. (703)305-3230

Authorized officer
Marianne Seidel

Telephone No. 703-308-1235

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

PCT/US02/35501

Continuation of B. FIELDS SEARCHED Item 3:
CAPLUS, USPATFUL, MEDLINE
search terms:aziridino compound, leukocytic nucleic acid, neoplastic disease, aziridinylquinone compound